

GeneChip™ HT WT Expression Assay Automated Workflow USER GUIDE

for use with:

WT Expression Kit, high throughput

GeneChip™ Whole Transcript (WT) Expression Arrays

Beckman Coulter™ Biomek™ FX^P Automated Workstation

Catalog Numbers 4440536 and 4440537

Publication Number MAN0018116

Revision B.0

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.



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WT Expression Kit, high throughput



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Products:

GeneChip™ Whole Transcript (WT) Expression Arrays

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Revision history: Pub. No. MAN0018116

Revision	Date	Description
B.0	2 December 2020	Add Bio-Rad™ white PCR plate, Cat. No. HSP9601, to the consumables list.
A.0	28 December 2018	- Initial release in Thermo Fisher Scientific document control system. - Supersedes legacy Affymetrix publication number 702923. - Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

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GeneChip™ HT WT Expression Assay Automated Workflow

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About the GeneChip™ HT WT Expression Assay

The GeneChip™ HT WT Expression Assay uses the Invitrogen™ WT Expression Kit, high throughput, in conjunction with the Applied Biosystems™ HT WT Terminal Labeling and Controls Kit to generate amplified and biotinylated sense-strand DNA targets from the entire expressed genome without bias. The assay and the associated reagents are for use with GeneChip™ ST arrays or GeneChip™ ST 1.1 array plates where “ST” stands for “Sense Target”. The probes on these arrays have been selected to be distributed throughout the entire length of each transcript.

Note: The HT WT Expression Assay is not compatible with GeneChip™ brand arrays designed to focus on the 3' ends of transcripts. For the 3' arrays, use the GeneChip™ HT 3' IVT Express Kit.

The WT Expression Kit enables you to prepare RNA samples for whole transcriptome (WT) analysis. The kit generates sense-strand cDNA from total RNA for subsequent fragmentation and labeling using the GeneChip™ WT Terminal Labeling and Controls Kit. It is optimized for use with human, mouse, or rat GeneChip™ Sense Target (ST) Arrays or Array Plates.

The WT Expression Kit represents the latest innovation in RNA amplification that enables faster, more sensitive, and highly reproducible results with whole transcriptome microarrays and array plates. This kit features:

- A streamlined workflow that requires as little as 50 ng of total RNA for a single round of amplification
- Direct amplification of total RNA without a separate rRNA depletion step
- A novel priming method that selectively reverse transcribes nonribosomal RNA for complete and unbiased coverage of the transcriptome
- Magnetic-bead cRNA purification for high recovery and ease of use
- Preformulated master mixes that significantly reduce hands-on time

The kit is based on a novel reverse transcription (RT) priming method that eliminates the need for a separate rRNA depletion step. The kit includes RT primers designed with a proprietary oligonucleotide matching algorithm that eliminates primer sequences with homology to known ribosomal RNA sequences. The result is complete and unbiased coverage of the transcriptome, with a significant reduction in rRNA amplification, compared to other methods.

The HT WT Expression Assay is developed for the Beckman Coulter™ Biomek™ FX^P Target Prep Express (TPE) System. This system uses robotic technology to automate many of the labor intensive tasks required when preparing eukaryotic RNA for whole transcriptome expression analysis.

Note: We do not recommend the use of user-prepared reagents for the HT WT Expression Assay. Use the GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates (array plates, Cat. No. 901622) or the Applied Biosystems™ GeneChip™ Hybridization, Wash, and Stain Kit (cartridge arrays, Cat. No. 900720) for all the reagents needed for the hybridization, wash and stain steps of this assay.

The Beckman Coulter™ Biomek™ FX^P TPE System automates the preparation of target that can be hybridized to a:

- GeneChip™ Cartridge Array that can be processed using the GeneChip™ Hybridization Oven (640 or 645), GeneChip™ Fluidics Station 450, and GeneChip™ Scanner 3000 System.
Target preparation for cartridge arrays includes 5 parts with an optional stopping point at the completion of each part (Figure 1). At the end of target preparation, the sample is ready to be denatured and hybridized onto a GeneChip™ ST Cartridge Array.
- GeneChip™ HT Array Plate and processed using the GeneTitan™ Multi-Channel (MC) Instrument.
Target preparation for array plates includes 6 parts for array plates (Figure 2) with an optional stopping point at the completion of each part. At the end of target preparation, the denatured sample is ready to be fragmented and hybridized onto a GeneChip™ ST Array Plate.

This manual describes the assay procedures recommended for eukaryotic target labeling for whole transcriptome expression analysis using the Beckman Coulter™ Biomek™ FX^P TPE System. The reagents and protocols have been developed and optimized specifically for use with the Beckman Coulter™ Biomek™ FX^P TPE System. It is anticipated that by following the recommended procedures, you will be able to generate sufficient biotin-labeled complementary DNA (cDNA target from each sample (8-, 16-, 24- or 96-sample workflow) for hybridization to a single GeneChip™ Cartridge or Array Plate. For information on RNA preparation, see the Chapter 2, “RNA preparation”.

Workflow of the HT WT Expression Assay

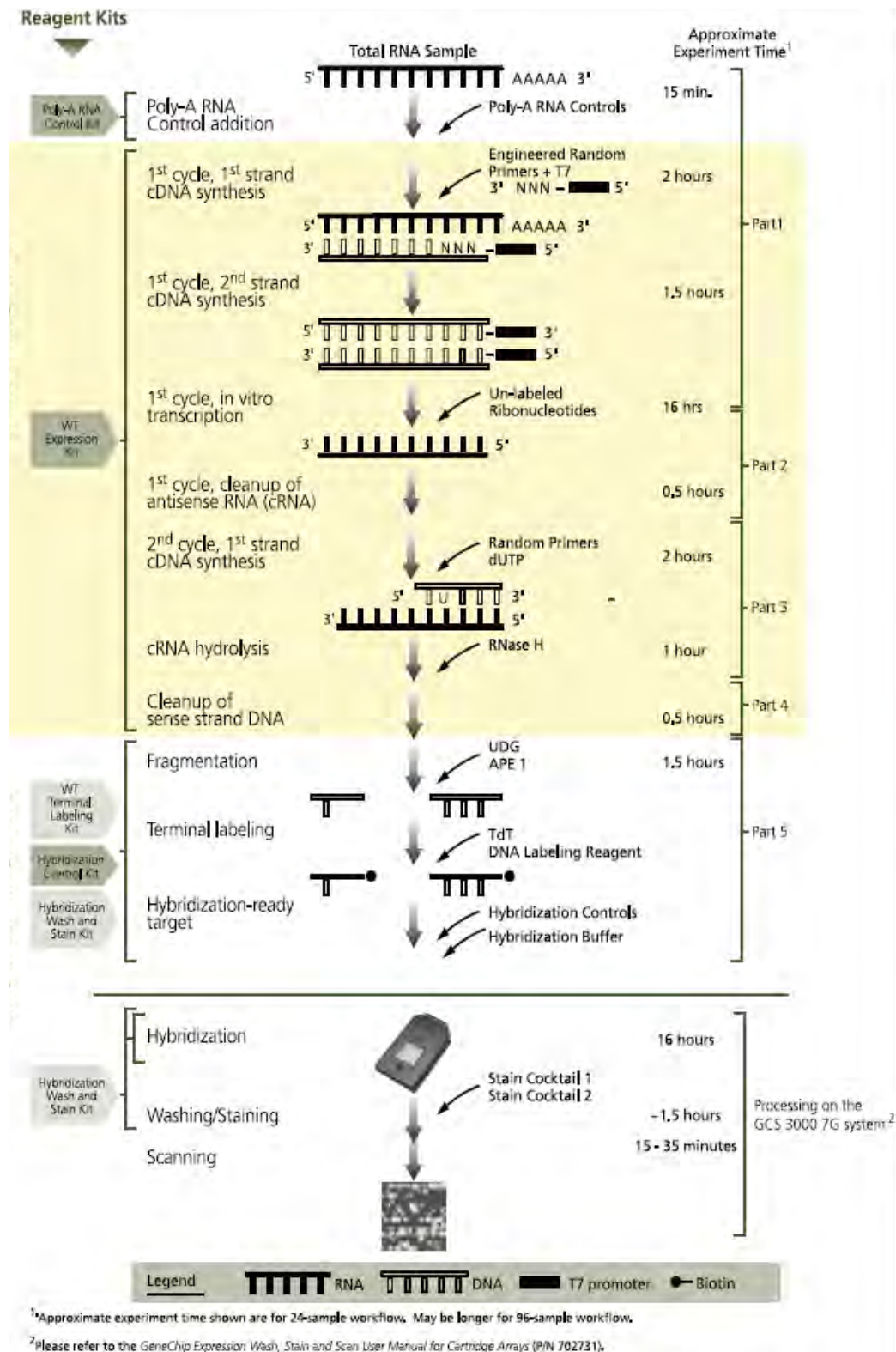


Figure 1 Overview of the HT WT Expression Assay for cartridge arrays.

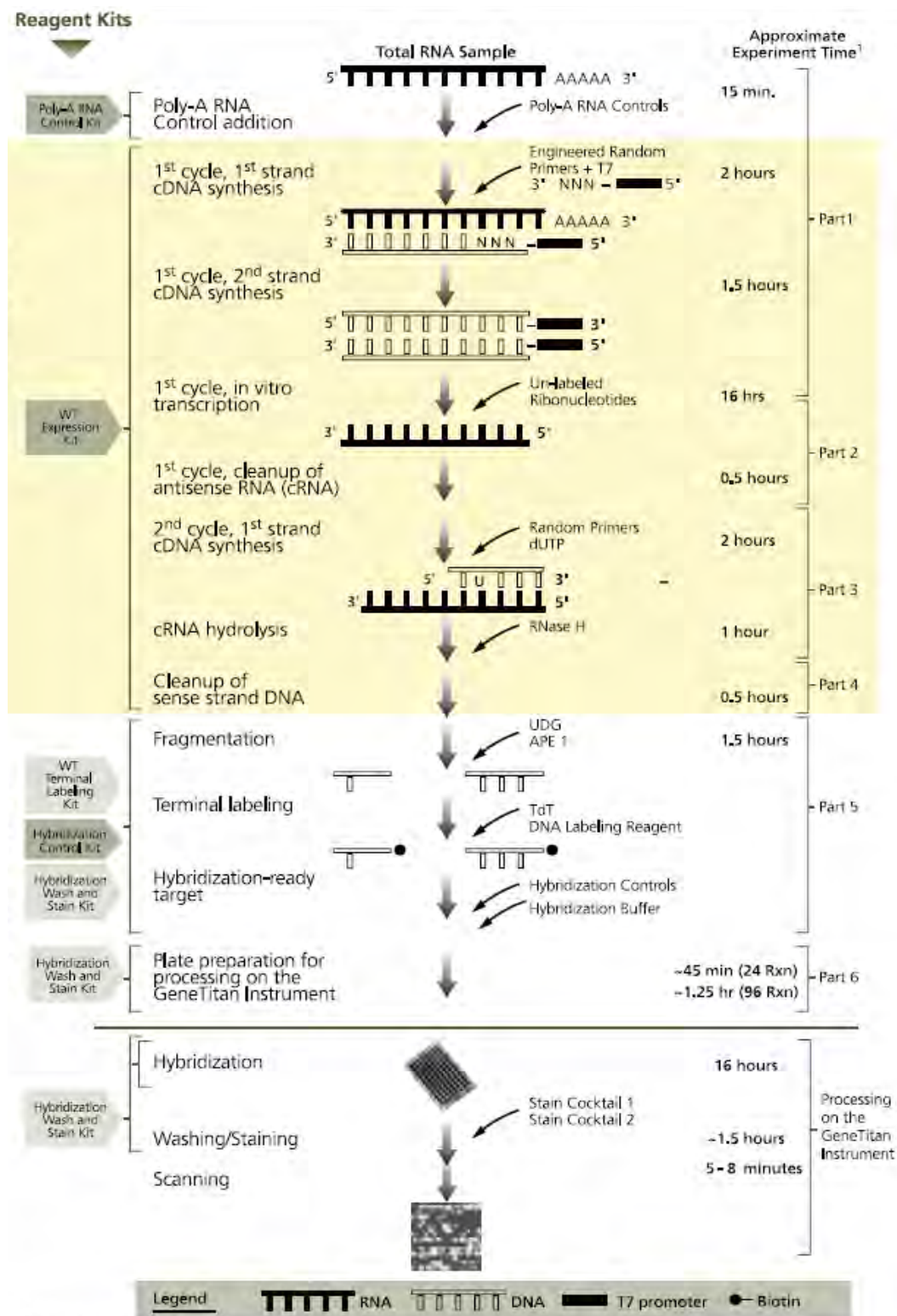


Figure 2 Overview of the HT WT Expression Assay for array plates.

Time required to perform the HT WT Expression Assay

The following tables provide the approximate time required to perform each part of the assay. Depending on the selected parameters, target preparation takes about 30 hours and 6 human user interventions. Additional time is required for hybridization, washing, staining, and scanning, and is dependent on the number of samples processed and the array type.

Approximate time required for Part 1

Part 1 — First- and Second-strand cDNA Synthesis, IVT cRNA Synthesis	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: first-strand synthesis	4	4	5	12
Thermal cycler: first strand method	120	120	120	120
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: second-strand synthesis	7	8	9	22
Thermal cycler: second-strand method	70	70	70	70
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: IVT	4	5	6	19
Thermal cycler: IVT method	960	960	960	960
Part 1 total time	1,180 ~19.7 hours	1,182 19.7 hours	1,185 19.75 hours	1,218 20.3 hours

Approximate time required for Part 2

Part 2 — cRNA Purification	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: cRNA purification	63	64	65	83
Normalization	3	3	3	11
Part 2 total time	81 1.35 hours	82 1.37 hours	83 1.38 hours	109 1.82 hours

Approximate time required for Part 3

Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	10	10	10	10
Biomek™ FXP Target Prep Express Automated Workstation pipetting steps: second-cycle denaturation	4	5	5	10
Thermal cycler: denaturation method	12	12	12	12
Biomek™ FXP Target Prep Express Automated Workstation pipetting steps: second-cycle synthesis	5	5	5	15
Thermal cycler: synthesis method	110	110	110	110
Biomek™ FXP Target Prep Express Automated Workstation pipetting steps: RNase H hydrolysis	4	5	5	11
Thermal cycler: RNase H method	50	50	50	50
Part 3 total time	195 3.25 hours	197 3.28 hours	197 3.28 hours	218 3.63 hours

Approximate time required for Part 4

Part 4—cDNA Purification	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FXP Target Prep Express Automated Workstation pipetting steps: cDNA purification	65	65	65	82
Normalization	4	4	4	11
Part 4 total time	84 1.40 hours	84 1.40 hours	84 1.40 hours	108 1.80 hours

Approximate time required for Part 5

Part 5—cDNA Fragmentation, Labeling, and Hybridization Setup	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	10	10	10	10
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: cDNA fragmentation	4	5	5	13
Thermal cycler: fragmentation method	64	64	64	64
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: labeling	4	5	5	13
Thermal cycler: labeling method	72	72	72	72
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: hybridization setup	10	11	11	13
GeneChip™ cartridge array hybridization setup	N/A	12	12	14
GeneChip™ array plate hybridization setup				
Part 5 total time for cartridge array	164 2.73 hours	167 2.78 hours	167 2.78 hours	185 3.08 hours
Part 5 total time for array plate	N/A	168 2.80 hours	168 2.80 hours	186 3.10 hours

Approximate time required for Part 6

Part 6—GeneTitan™ MC Instrument Hybridization Preparation	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	N/A	10	10	10
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps	N/A	10	30	60
Thermal cycler: denaturation method	N/A	12	12	12
Part 6 total time	N/A	32	52	82 1.37 hours



RNA preparation

■ Quantitation of RNA	14
■ Preparation of poly-A RNA controls	15
■ Preparation of total RNA plates for processing on automated systems	17

This chapter describes the general requirements for starting total RNA sample, poly-A control preparation for spiking into your RNA sample, and preparation of total RNA plates for processing on automated systems.

IMPORTANT! The quality of the RNA is essential to the overall success of the analysis. Since the most appropriate protocol for the isolation of RNA can be source dependent, we recommend using a protocol that has been established for the tissues or cells being used. In the absence of an established protocol, using one of the commercially available kits designed for RNA isolation is suggested

When using a commercial kit, follow the manufacturer's instructions for RNA isolation.

Quantitation of RNA

Quantify RNA yield by spectrophotometric analysis using the convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA.

- The absorbance should be checked at 260 and 280 nm for determination of sample concentration and purity.
- The A_{260}/A_{280} ratio should be close to 2.0 for pure RNA (ratios between 1.7 and 2.1 are acceptable).
- Integrity of total RNA samples can also be assessed qualitatively on an Agilent™ 2100 Bioanalyzer™. See Figure 3 for an example of good-quality total RNA sample.

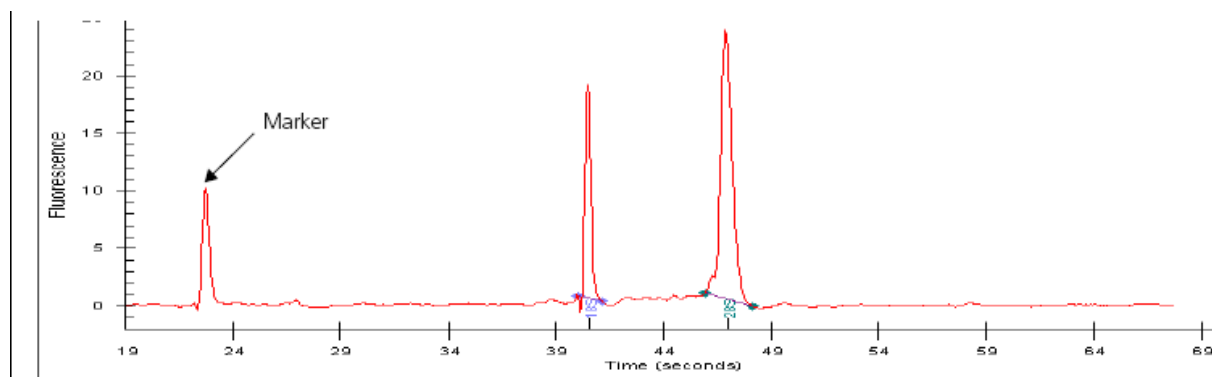


Figure 3 Electropherogram (from the Agilent™ 2100 Bioanalyzer™) for HeLa total RNA. For a high-quality total RNA sample, 2 well-defined peaks corresponding to the 18S and 28S ribosomal RNAs should be observed, similar to a denaturing agarose gel, with ratios approaching 2:1 for the 28S to 18S bands.

Preparation of poly-A RNA controls

Reagents and equipment

- Invitrogen™ WT Expression Kit, high throughput, Cat. No. [4440537](#) (1 x 24 reactions) or Cat. No. [4440536](#) (1 x 96 reactions).
- GeneChip™ HT WT Terminal Labeling and Controls Kit, Cat. No. [901647](#) (24 reactions) or Cat No. [901648](#) (96 reactions).

Bundle Includes:

- GeneChip™ HT WT Terminal Labeling Kit
- GeneChip™ Hybridization Control Kit
- GeneChip™ Eukaryotic Poly-A RNA Control Kit

Components used in this step:

- Eukaryotic Poly-A RNA Controls

Designed specifically to provide exogenous positive controls to monitor the entire eukaryotic target labeling process, a set of poly-A RNA controls is supplied in the GeneChip™ Eukaryotic Poly-A RNA Control Kit.

Each eukaryotic GeneChip™ probe array contains probe sets for several *B. subtilis* genes that are absent in eukaryotic samples (*lys*, *phe*, *thr*, and *dap*). These poly-A RNA controls are *in vitro* synthesized, and the polyadenylated transcripts for the *B. subtilis* genes are premixed at staggered concentrations. The concentrated Poly-A Control Stock can be diluted with the Poly-A Control Dil Buffer and spiked directly into RNA samples to achieve the final dilutions (relative to estimated copy number of total mRNA population) summarized in Table 1.

Table 1 Final dilutions of Poly-A Control StockPoly-A RNA controls in samples.

Poly-A RNA spike	Final dilution (estimated ratio of copy number)
<i>lys</i>	1:100,000
<i>phe</i>	1:50,000
<i>thr</i>	1:25,000
<i>dap</i>	1:6,667

The controls are then amplified and labeled together with the samples. Examining the hybridization intensities of these controls on GeneChip™ arrays helps to monitor the labeling process independently from the quality of the starting RNA samples. Anticipated relative signal strength follows the order of *lys* < *phe* < *thr* < *dap*.

The Poly-A Control Stock and Poly-A Control Dil Buffer are provided in the GeneChip™ Eukaryotic Poly-A RNA Control Kit to prepare the appropriate serial dilutions based on Table 2. This is a guideline when 100, 250, or 500 ng of total RNA is used as starting material. For starting sample amounts other than those listed here, calculations are needed in order to perform the appropriate dilutions to arrive at the same proportionate final concentration of the spike-in controls in the samples.

Table 2 Serial dilution of Poly-A Control Stock.

Total RNA input amount	Serial dilutions				Volume of fourth dilution to add total RNA
	First dilution	Second dilution	Third dilution	Fourth dilution	
100 ng	1:20	1:50	1:50	1:10	2 µL
250 ng	1:20	1:50	1:50	1:4	2 µL
500 ng	1:20	1:50	1:50	1:2	2 µL

IMPORTANT! Use nonstick RNase-free microfuge tubes to prepare all of the dilutions (not included).

Recommendation: Avoid pipetting solutions less than 2 µL in volume to maintain precision and consistency when preparing the dilutions.

Example to prepare the poly-A RNA dilutions for 100 ng of total RNA

1. Add 2 µL of the Poly-A Control Stock to 38 µL of Poly-A Control Dil Buffer for the first dilution (1:20).
2. Mix thoroughly and centrifuge to collect the liquid at the bottom of the tube.
3. Add 2 µL of the first dilution to 98 µL of Poly-A Control Dil Buffer to prepare the second dilution (1:50).
4. Mix thoroughly and centrifuge to collect the liquid at the bottom of the tube.

5. Add 2 μL of the second dilution to 98 μL of Poly-A Control Dil Buffer to prepare the third dilution (1:50).
6. Mix thoroughly and centrifuge to collect the liquid at the bottom of the tube.
7. Add 2 μL of this third dilution to 18 μL of Poly-A Control Dil Buffer to prepare the fourth dilution (1:10).

Note: The first dilution of the poly-A RNA controls can be stored up to 6 weeks in a frost-free freezer at -20°C and freeze-thawed up to 8 times.

8. Mix thoroughly and centrifuge to collect liquid at the bottom of tube.
9. Add 2 μL of this fourth dilution to 100 ng of Total RNA directly. The final volume of Total RNA with the diluted poly-A controls should not exceed 5 μL .

Preparation of total RNA plates for processing on automated systems

The Target Preparation protocol starts with 5 μL of material in a Bio-Rad™ 96-Well Hard-Shell™ PCR Plate. This plate can be prepared either offline (manually) or on the Beckman Coulter™ Biomek™ FX^P Target Prep Express (TPE) System, as described in the following topics.

User-prepared plate preparation

Preparing samples with poly-A controls

Pipet 3 μL of the total RNA sample (100 - 500 ng) and 2 μL of the appropriate PolyA spike control solution into the 96-well plate. Samples should be placed into the plate in a column-wise fashion starting from the left side of the plate. For example, if preparing 24 samples, pipet the samples into the sample wells for columns 1, 2, and 3.

Optional—running without poly-A controls

We highly recommend the use of poly-A controls as described previously. However, if controls are not used, the total RNA sample in the Bio-Rad™ 96-Well Hard-Shell™ PCR Plate must be adjusted to a final volume of 5 μL .

3

Labware and reagents required

■ Equipment, consumables, labware, and reagents required	18
■ Labware	21
■ Reagent and labware requirements for Part 1 to Part 6	27

This chapter provides a check list of required labware and reagents.

Equipment, consumables, labware, and reagents required

Equipment and labware required

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 3 Beckman Coulter™ Biomek™ FX^P Hybrid Thermo Target Prep Workstation.

Product	Quantity	Source
Beckman Coulter™ Biomek™ FX ^P Hybrid Thermo Target Prep Workstation	1	Beckman Coulter™, A83103

Table 4 Applied Biosystems™ Expression ATP Install Kit for Beckman Biomek™ FX^P Target Prep Express.

Component	Quantity	Source
Applied Biosystems™ Expression ATP Install Kit for Beckman Biomek™ FX ^P Target Prep Express. Installation kit consists of:		90-0931
HT WT Reservoir Stickers	1 set	
HT WT Cold Block Template	1	
Alpaqua™ Magnetic Plate	1	
Alpillo™ Plate Cushion	1	
WT ATP Biomek™ method file (and support files)	Installed by FAS	
Arched Auto-Sealing Lids with Wide Tabs	Package (package of 4)	

Table 5 Applied Biosystems™ HT WT Expression Assay Consumables for Biomek™ FX^P Target Prep Express.

Component	Number per kit	Source
HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express Labware sufficient for 4 x 24 or 4 x 96 reaction runs consists of:		901646
96-Well Deepwell Microplate, polypropylene, 2 mL, nonsterile	8	
Greiner Bio-One™ U-bottom (round) Plate, polypropylene	8	
Bio-Rad™ Hard-Shell™ PCR Plate, blue shell/clear well	72	
Greiner Bio-One™ UV-Star™ Plate, nonsterile, clear, flat bottom	8	
Costar™ 96-Well U-bottom plate, polystyrene	16	
24-Well Plate, 10 mL, square well, pyramid bottom, polypropylene	4	
Bio-Rad™ Microseal™ 'P' Reusable Replacement PCR Plate Sealing Pads	2	

Table 6 Biomek™ tips and universal labware.

Product	Packaging	Total 8 reactions	Total 16 reactions	Total 24 reactions	Total 96 reactions	Source
Biomek™ Span P50 Barrier Tips (Span-8 Pipettor) (magenta)	96 each/box 10 box/case	10 boxes	10 boxes	10 boxes	10 boxes	A21586
Biomek™ AP96—P250 Barrier Tips Sterile (AP 96 Multichannel Pipettor) (aqua)	96 each/box 10 box/case	8 boxes	8 boxes	8 boxes	8 boxes	717253
Biomek™ Span P250 Barrier Tips (Span-8 Pipettor) (green)	96 each/box 10 box/case	1 Box	1 Box	1 Box	1 Box	379503
Biomek™ Span P1000 LLS Barrier Tips (Span-8 Pipettor) (yellow)	96 each/box 5 box/case	2 boxes	2 boxes	2 boxes	2 boxes	987925
Quarter Reservoir, Divided by Width	40 unit/case	4	4	4	4	372792
Quarter Reservoir	40 unit/case	8	8	8	8	372790
Frame for Reservoirs	1 each	reusable		reusable		372795

Reagents required

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Table 7 HT WT express reagents, miscellaneous reagents, and materials.

Product	Source
Invitrogen™ WT Expression Kit, high throughput. Packaging options: 1 x 24 reaction kit 1 x 96 reaction kit	4440536 4440537

Table 7 HT WT express reagents, miscellaneous reagents, and materials. (continued)

Product	Source
Applied Biosystems™ HT WT Terminal Labeling and Controls Kit ^[1] (sufficient for 24 reactions). Kit contains: - GeneChip™ HT WT Terminal Labeling Kit, Cat. No. 901555 (1 x 24 reaction kit) - GeneChip™ Hybridization Control Kit, Cat. No. 900454 (30 reactions) - GeneChip™ Eukaryotic Poly-A RNA Control Kit, Cat. No. 900433 (100 reactions)	901647
Applied Biosystems™ HT WT Terminal Labeling and Controls Kit ^[1] (sufficient for 96 reactions). Kit contains: - GeneChip™ HT WT Terminal Labeling Kit, Cat. No. 901518 (1 x 96 reaction kit) - GeneChip™ Hybridization Control Kit, Cat. No. 900457 (150 reactions) - GeneChip™ Eukaryotic Poly-A RNA Control Kit, Cat. No. 900433 (100 reactions)	901648
Applied Biosystems™ GeneChip™ Hybridization, Wash, and Stain Kit (30 reactions, for cartridges)	900720
Applied Biosystems™ GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates (96 reactions)	901622
DNAZap™ PCR DNA Degradation Solutions	AM9890
RNaseZap™ RNase Decontamination Wipes	AM9786
100% ETOH	MLS
Isopropanol	MLS

^[1] Individual components available for purchase separately.

Labware

The following table lists and describes the types of labware used in the GeneChip™ HT WT Expression Assay. For labware requirements specific to each part of the assay, see “Reagent and labware requirements for Part 1 to Part 6” on page 27.

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 8 Labware used on the Biomek™ FX^P Target Prep Express Automated Workstation deck.

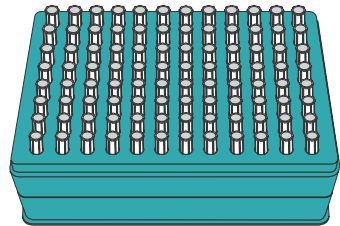
Labware	Source	Labware image
Biomek™ AP96–P250 Barrier Tips (aqua box; presterile, barrier)	Beckman Coulter™, 717253	

Table 8 Labware used on the Biomek FXP Target Prep Express Automated Workstation deck. (continued)

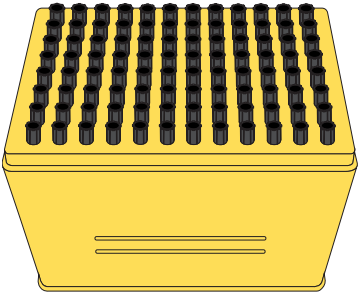
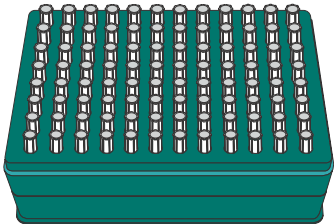
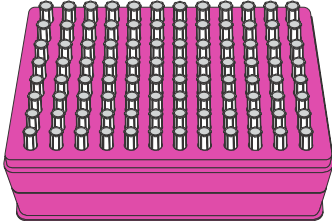
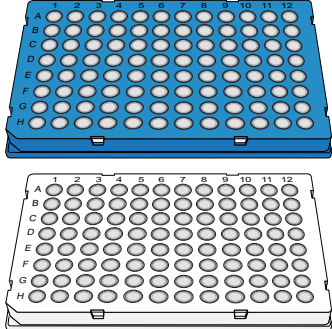
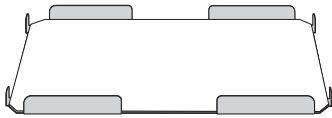
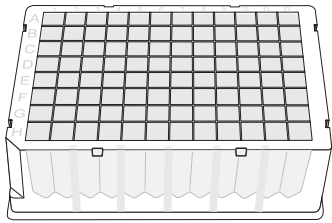
Labware	Source	Labware image
Biomek™ Span P1000 LLS Barrier Tips (yellow box; presterile, barrier, conductive)	Beckman Coulter™, 987925	
Biomek™ Span P250 Barrier Tips (green box; presterile, barrier)	Beckman Coulter™, 379503	
Biomek™ Span P50 Pipette Tips (magenta box; presterile, barrier)	Beckman Coulter™, A21586	
Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (available in multiple colors)	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646. Kit = 72 or Bio-Rad™, HSP9631 (blue) or HSP9601 (white)	
Bio-Rad™ Arched Auto-Sealing Lids With Wide Tabs for PCR Plates	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646. Kit = 2 or Bio-Rad Laboratories™, MSL2032	
ABgene™ 96 Square Well Storage Plate (2.2 mL)	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646. Kit = 8 or ABgene™, AB-0932	

Table 8 Labware used on the Biomek FXP Target Prep Express Automated Workstation deck. (continued)

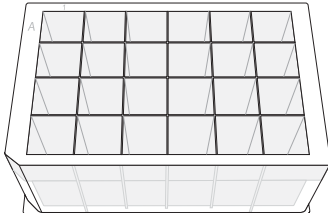
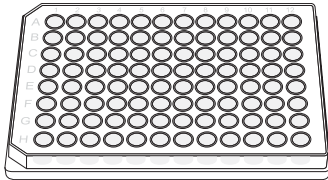
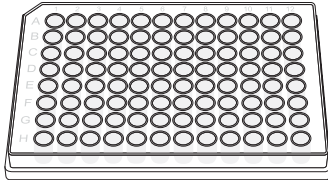
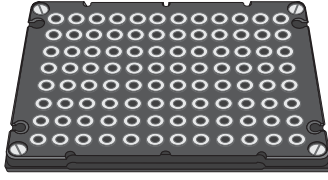
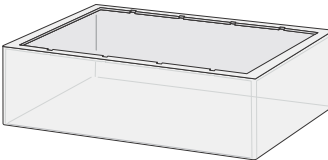
Labware	Source	Labware image
Agilent™ 24-well mixing plate, 10 mL, square well, pyramid bottom	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646 . Kit = 4 or Thomas Scientific™, EK-2053	
Greiner Bio-One™ UV-Star™ Plate	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646 . Kit = 8 or Greiner Bio-One™, 655801	
Greiner Bio-One™ 96-well U-bottom (round) plate, polypropylene	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646 . Kit = 8 or Greiner Bio-One™, 650201	
Costar™ Polystyrene 96-well, U-bottom Plate (nonsterile)	A component of the HT WT Expression Assay Consumables for Biomek™ FX ^P Target Prep Express, 901646 . Kit = 16 or Costar™, 3798	
Alpaqua™ Magnetic Plate	A component of the Expression ATP Install Kit for Beckman Biomek™ FX ^P Target Prep Express, 90-0931	
Reservoir Frame	Beckman Coulter™, 372795	

Table 8 Labware used on the Biomek FXP Target Prep Express Automated Workstation deck. (continued)

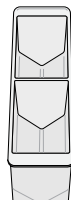
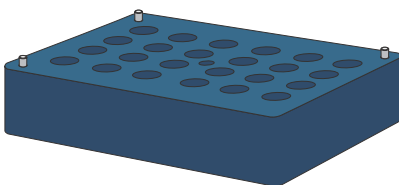
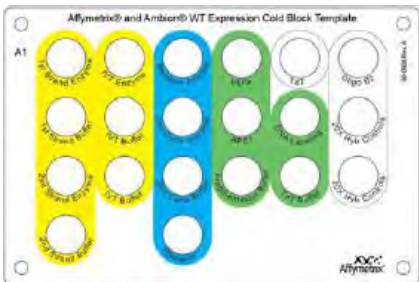
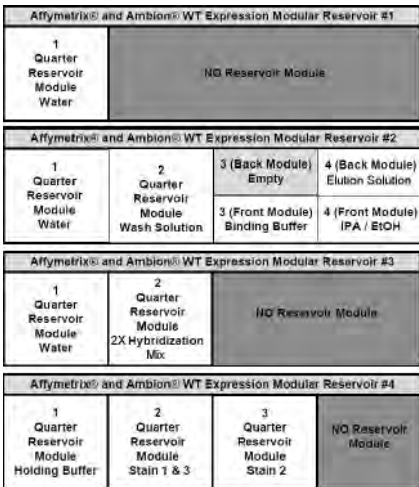
Labware	Source	Labware image	
Quarter Reservoirs - Quarter module, 40 mL capacity - Quarter module divided by width, 19 mL capacity each receptacle	Beckman Coulter™ 372790 (40 mL) 372792 (19 mL)	Undivided 40 mL capacity 	Divided by width 19 mL capacity 
Reagent Cold Block, must be chilled to 4°C before use	Beckman Coulter™, A83054		
Reagent Cold Block Template The template is designed specifically for use with the GeneChip™ HT WT Reagent Kit and fits precisely onto the top of the chilled cold block. Using the cold block template helps ensure the proper placement of reagent tubes onto the block for each part of the assay.	90-0906 A component of the Expression ATP Install Kit for Beckman Biomek™ FX ^P Target Prep Express, 90-0931		
Modular Reservoir Template Stickers Modular reservoir template stickers are available to place on the front of the modular reservoir frame to, assist with the proper placement of modular reservoirs and reagents. See Figure 5 for further detail.	90-0907 A component of the Expression ATP Install Kit for Beckman Biomek™ FX ^P Target Prep Express, 90-0931		

Table 8 Labware used on the Biomek FXP Target Prep Express Automated Workstation deck. *(continued)*

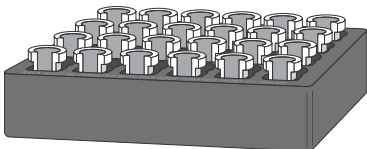
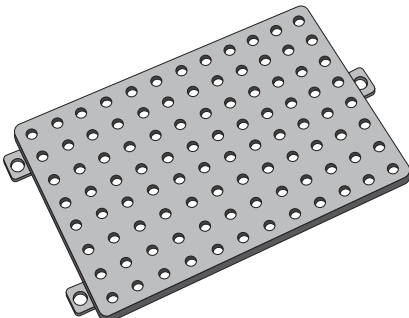
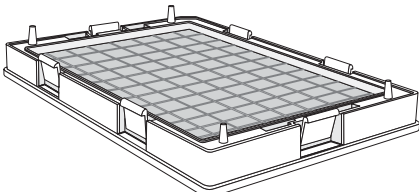
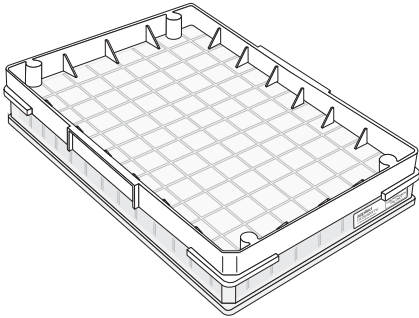
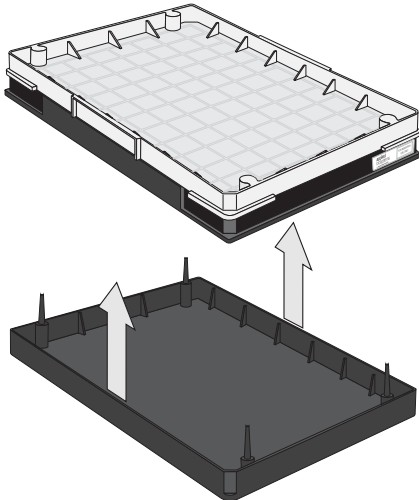
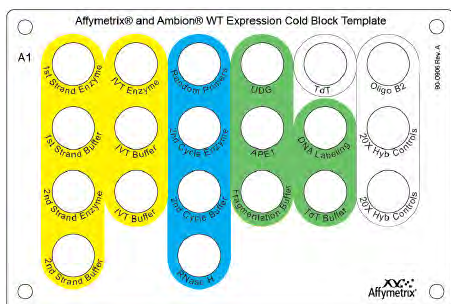
Labware	Source	Labware image
24-Position Tube Rack with 24 inserts	Beckman Coulter™ 373661 (rack) 373696 (insert)	
Adaptor, Thin PCR (installed on the Shaking Peltier) This adaptor is typically installed by a Beckman Coulter™ field service technician during new system installation or a system upgrade. Ensure that you have one of these adaptors on the deck prior to running this assay.	Beckman Coulter™, A19709	
Alpillo™ Plate Cushion	A component of the Expression ATP Install Kit for Beckman Biomek™ FX ^P Target Prep Express, 90-0931	
GeneTitan™ Hybridization Tray	500867 GeneTitan™ trays and lids are provided with the array plate when ordered. The Cat. No. provided here is for identification purposes only.	
GeneTitan™ Stain Tray	501025 GeneTitan™ trays and lids are provided with the array plate when ordered. The Cat. No. provided here is for identification purposes only.	

Table 8 Labware used on the Biomek FXP Target Prep Express Automated Workstation deck. *(continued)*

Labware	Source	Labware image
GeneTitan™ Scan Tray	501006 or 500860 GeneTitan™ trays and lids are provided with the array plate when ordered. The Cat. No. provided here is for identification purposes only.	

Labware templates

The Expression ATP Install Kit for Beckman Biomek™ FX^P Target Prep Express provides templates to help ensure the proper placement of reagents in the labware.

**Figure 4** Reagent cold block template.

Use Template #1 for:

- Part 1
- Part 3
- Part 5 - Array plates only

Affymetrix® and Ambion® WT Expression Modular Reservoir #1	
1 Quarter Reservoir Module Water	NO Reservoir Module

Use Template #2 for:

- Part 2
- Part 4

Affymetrix® and Ambion® WT Expression Modular Reservoir #2			
1 Quarter Reservoir Module Water	2 Quarter Reservoir Module Wash Solution	3 (Back Module) Empty	4 (Back Module) Elution Solution
		3 (Front Module) Binding Buffer	4 (Front Module) IPA / EtOH

Use Template #3 for:

- Part 5 - Cartridge arrays only

Affymetrix® and Ambion® WT Expression Modular Reservoir #3	
1 Quarter Reservoir Module Water	2 Quarter Reservoir Module 2X Hybridization Mix
NO Reservoir Module	

Use Template #4 for:

- Part 6

Affymetrix® and Ambion® WT Expression Modular Reservoir #4			
1 Quarter Reservoir Module Holding Buffer	2 Quarter Reservoir Module Stain 1 & 3	3 Quarter Reservoir Module Stain 2	NO Reservoir Module

Figure 5 Modular reservoir template stickers.

Reagent and labware requirements for Part 1 to Part 6

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Reagents and labware required for Part 1

Do the following when instructed to thaw, vortex, and centrifuge buffer reagent tubes. For enzyme reagent tubes, flick the tubes and centrifuge only.

- Vortex reagents—3 times, 1 second each time at the maximum setting.
- Centrifuge reagent vials—3 seconds



CAUTION! Carefully observe the buffer tubes after vortexing. If a precipitant is present, additional vortexing is required. Vortex until all precipitant is dissolved. Heat at 37 to 40°C if necessary.

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 9 Part 1 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 (24 reactions) ^[1] or 4440536 (96 reactions) Components needed: • 1st Strand Enzyme Mix • 1st Strand Buffer Mix • 2nd Strand Enzyme Mix • 2nd Strand Buffer Mix • IVT Enzyme Mix • IVT Buffer Mix
	Nuclease-free Water
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: To use a 24-reaction WT Expression Kit for two 8-sample runs, see Appendix A, “Using the 24-reaction kit for two 8-sample runs”.

Table 10 Part 1 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
1	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
1 box	1 box	1 box	1 box	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253

Table 10 Part 1 labware requirements. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
1	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Reagents and labware required for Part 2

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 11 Part 2 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput, 4440537, 24 reactions^[1] or 4440536, 96 reactions. Components needed: • Nucleic Acid Binding Buffer Concentrate • Nucleic Acid Binding Beads • Nucleic Acid Wash Concentrate • Elution Solution • Nuclease-free Water • Isopropanol • Ethanol, 200 proof

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: All components and reagents, except beads, for Part 2 and Part 4 should be at room temperature. Allow samples to sit at room temperature for at least 10 minutes. The Bead Binding Buffer will precipitate if stored at 4°C. Ensure that the binding buffer and beads are at room temperature prior to starting the procedure.

Table 12 Part 2 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ AP96 – P250 Barrier Tips (aqua) Beckman Coulter™, 717253
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
2	2	2	2	Costar™ U-bottom 96-well, Polystyrene Plate Costar™, 3798, or part of 901646
1	1	1	2	ABgene™ 96 Square Well Storage Plate ABgene™, AB-0932, or part of 901646
1	1	1	2	Greiner Bio-One™ UV-Star™ Plate Greiner Bio-One™, 655801, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2453, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790

Table 12 Part 2 labware requirements. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
2	2	2	2	Quarter Reservoir Divided by Width Beckman Coulter™, 372792
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Reagents and labware required for Part 3

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 13 Part 3 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 , 24 reactions ^[1] or 4440536 , 96 reactions Components needed: • Random Primers • Second-Cycle Enzyme Mix • Second-Cycle Buffer Mix • RNase H • Nuclease-free Water

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Table 14 Part 3 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
0 tips	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	0 tips	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586

Table 14 Part 3 labware requirements. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™ HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
1	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Reagents and labware required for Part 4

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 15 Part 4 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 , 24 reactions ^[1] or 4440536 , 96 reactions Components needed: • Nucleic Acid Binding Buffer Concentrate • Nucleic Acid Binding Beads • Nucleic Acid Wash Solution Concentrate • Elution Solution • Nuclease-free Water • Ethanol, 200 proof
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: All components and reagents, except beads, for Part 2 and Part 4 should be at room temperature. Allow samples to sit at room temperature for at least 10 minutes. The Bead Binding Buffer will precipitate if stored at 4°C. Ensure that the binding buffer and beads are at room temperature prior to starting the procedure.

Table 16 Part 4 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™ HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
2	2	2	2	Costar™ U-bottom 96-well, Polystyrene Plate Costar™, 3798, or part of 901646
1	1	1	1	ABgene™ 96 Square Well Storage Plate ABgene™, AB-0932, or part of 901646
1	1	1	1	Greiner Bio-One™ UV-Star™ Plate Greiner Bio-One™, 655801, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790

Table 16 Part 4 labware requirements. *(continued)*

8 reactions	16 reactions	24 reactions	96 reactions	Description
2	2	2	2	Quarter Reservoir Divided by Width Beckman Coulter™, 372792
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Reagents and labware required for Part 5

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 17 Part 5 reagent requirements.

Per run	Description
1	GeneChip™ HT WT Terminal Labeling Kit 901555 (24 reactions ^[1]), 901518 (96 reactions) Components needed: • 10X cDNA Fragmentation Buffer • UDG, 10 U/uL • APE 1, 1000 U/uL • 5X TdT Buffer • TdT, 30 U/uL • DNA Labeling Reagent, 5 mM • RNase-free Water
see Table 18	GeneChip™ Hybridization Control Kit 900454 (30 reactions) or 900457 (150 reactions)
see Table 18	GeneChip™ Hybridization, Wash, and Stain Kit, 900720 (30 reactions) or GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates, 901622 (96 reactions)
	Nuclease-free Water
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Table 18 Part 5 kit requirements.

Sample format				
	8 ^[1] , 16, and 24 reactions		96 reactions	
Array option:	Cartridge	Plates	Cartridge	Plates
Hybridization, wash, and stain kit	30 reactions 900720	96 reactions 901622	4 x 30 reactions 900720	96 reactions 901622
Hybridization control kit	30 reactions 900454	30 reactions 900454	150 reactions 900457	150 reactions 900457

^[1] Cartridge option only for 8-sample reaction.

Table 19 Part 5 labware requirements for cartridges.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
1	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
3	3	3	3	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931

Table 19 Part 5 labware requirements for cartridges. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Greiner Bio-One™ U-Bottom 96 well Polypropylene Plate Greiner Bio-One™, 650201, or part of 901646
1	1	1	1	24-Position Tube Rack Beckman Coulter™, 373661 (24-position rack), 373696 (white 10-mm insert)
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Table 20 Part 5 labware requirements for plates.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
N/A	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
N/A	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
N/A	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
N/A	3 boxes	3 boxes	3 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253

Table 20 Part 5 labware requirements for plates. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	6	6	6	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
N/A	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
N/A	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
N/A	1	1	1	24-Position Tube Rack Beckman Coulter™, 373661 (24-position rack), 373696 (white 10-mm insert)
N/A	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
N/A		1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
N/A	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
N/A	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Reagents and labware required for Part 6

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 21 Part 6 reagent requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	√	√	√	Hybridization-ready target plate (from Part 5)
N/A	1	1	1	GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates, 901622 Components needed: • Array Holding Buffer • Stain 1 & 3 • Stain 2

Table 22 Part 6 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	1 box	1 box	1 box	Biomek™ Span™ P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
N/A	1 box	1 box	1 box	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
N/A	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™ MSL-2032, or part of 901646
N/A	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
N/A	1	1	1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
N/A	1	1	1	GeneTitan™ Hybridization Tray, 500867 ^[1]
N/A	3	3	3	GeneTitan™ Stain Tray, 501025 ^[1]
N/A	1	1	1	GeneTitan™ Scan Tray, 501006 or 500860 ^[1]
N/A	4	4	4	GeneTitan™ Stain/Scan Tray Lids, 202757 ^[1]
N/A	3	3	3	Quarter Reservoir Beckman Coulter™, 372790
N/A	1	1	1	Reservoir Frame Beckman Coulter™, 372795

^[1] GeneTitan™ trays and lids are provided with the array plate when ordered. The Cat. No. provided here is for identification purposes only.



Biomek™ FX^P Automated Workstation setup and target preparation

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This chapter explains how to perform WT expression and terminal labeling using the Biomek™ FX^P Target Prep Express (TPE) System. It also includes instructions for target preparation for processing on the GeneTitan™ Instrument. The protocol is performed on 2 instruments:

- Target preparation is performed on the Biomek™ FX^P Target Prep Express Automated Workstation.
- Array processing is performed on the GeneTitan™ MC Instrument.
See the *GeneTitan™ Instrument User Guide for Expression Array Plates* for instruction on processing array plates using the GeneTitan™ MC Instrument.

Part 1—First- and Second-strand cDNA Synthesis, IVT cRNA Synthesis

This reaction synthesizes single-stranded cDNA. The cDNA is then converted to double-stranded cDNA, which acts as a template for *in vitro* transcription. At completion, Part 1 yields a Bio-Rad™ Hard-Shell™ 96-Well PCR Plate with unpurified cRNA.

Approximate time required for Part 1

Part 1—First- and Second-strand cDNA Synthesis, IVT cRNA Synthesis	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: first-strand synthesis	4	4	5	12
Thermal cycler: first strand method	120	120	120	120
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: second-strand synthesis	7	8	9	22
Thermal cycler: second-strand method	70	70	70	70
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: IVT	4	5	6	19
Thermal cycler: IVT method	960	960	960	960
Part 1 total time	1,180 ~19.7 hours	1,182 19.7 hours	1,185 19.75 hours	1,218 20.3 hours

Reagents and labware required for Part 1

Do the following when instructed to thaw, vortex, and centrifuge buffer reagent tubes. For enzyme reagent tubes, flick the tubes and centrifuge only.

- Vortex reagents—3 times, 1 second each time at the maximum setting.
- Centrifuge reagent vials—3 seconds



CAUTION! Carefully observe the buffer tubes after vortexing. If a precipitant is present, additional vortexing is required. Vortex until all precipitant is dissolved. Heat at 37 to 40°C if necessary.

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 23 Part 1 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 (24 reactions) ^[1] or 4440536 (96 reactions) Components needed: • 1st Strand Enzyme Mix • 1st Strand Buffer Mix • 2nd Strand Enzyme Mix • 2nd Strand Buffer Mix • IVT Enzyme Mix • IVT Buffer Mix
	Nuclease-free Water
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: To use a 24-reaction WT Expression Kit for two 8-sample runs, see Appendix A, “Using the 24-reaction kit for two 8-sample runs”.

Table 24 Part 1 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
1	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
1 box	1 box	1 box	1 box	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253

Table 24 Part 1 labware requirements. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
1	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Clean the 'P' Pad in the autosealing microplate lid

The reusable/disposable Microseal™ 'P' Pad under the arched autosealing microplate lid should be cleaned before every run and replaced every 25 runs.

Materials required

- DNAZap™ PCR DNA Degradation Solutions
- RNaseZap™ RNase Decontamination Solution or RNaseZap™ RNase Decontamination Wipes
- Laboratory wipes

Clean the Microseal™ 'P' Pad

1. Rinse the pad with deionized water.
2. Wipe the pad with RNaseZap™ RNase Decontamination Wipes.
3. Rinse the pad with deionized water.
4. Wipe the pad with DNAZap™ PCR DNA Degradation Solutions.

5. Thoroughly rinse the pad with deionized water.
6. Dry the pad with pressurized clean air or nitrogen.

Prepare the Biomek™ FX^P TPE System

Before beginning a sample preparation run, make the following checks of the Biomek™ FX^P TPE System.

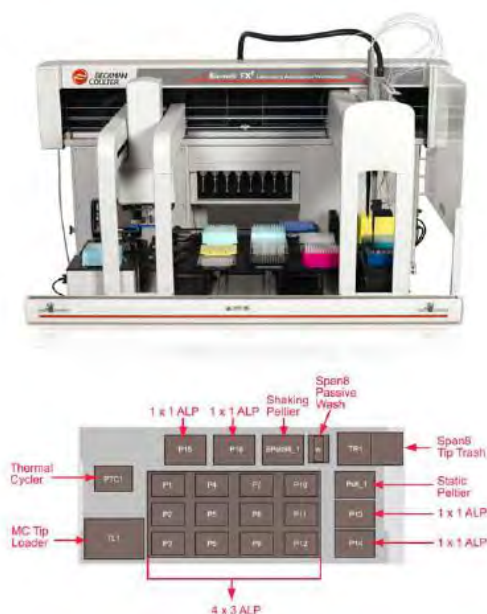


Figure 6 Biomek™ FX^P TPE System.

Check the water and waste containers

1. If necessary, fill the system water container using distilled, deionized water (ultra-pure water not required).
2. If necessary, empty the system waste bottle.
3. If necessary, empty the tip waste container.

Turn on the Biomek™ FX^P Target Prep Express Automated Workstation

1. Power on the workstation.
2. Ensure that all of the peripherals are powered on.
 - Watlow temperature controllers control the Static Peltier (Pelt_1) and the Shaking Peltier (SPelt_96); no additional power supply.
 - Thermal cycler:
 - Bio-Rad™ DNA Engine™ PTC-200 Thermal Cycler, or
 - Whatman™ Biometra™ TRobot 96 Thermocycler



CAUTION! If using a DNA Engine™ PTC-200 Thermal Cycler, before turning it on confirm that the multichannel head (pod_1) is not positioned over the thermal cycler.

3. Launch the Biomek™ Software by double-clicking the Biomek™ Software icon on the desktop . You can also open **Start ▶ All Programs ▶ Beckman Coulter ▶ Biomek Software**.

Note: The instructions that follow pertain to the DNA Engine™ PTC-200 Thermal Cycler. If using a Biometra™ TRobot 96 Thermocycler proceed to “Home all axes” on page 46.

Note: For detailed instructions on how to use either the DNA Engine™ PTC-200 Thermal Cycler or the Biometra™ TRobot 96 Thermocycler, see the appropriate Beckman Coulter™ user manual for your specific thermal cycler.

Close the DNA Engine™ PTC-200 Thermal Cycler lid

The DNA Engine™ PTC-200 Thermal Cycler may remain open upon startup. You must close the lid prior to homing the axes or starting a method.

Be sure to close the PTC-200 lid if the lid:

- Remains open after you have powered on the workstation.
- Is up before you home the axes or before you begin a method.

If not closed, the MC Pod may collide with the lid and damage the instrument.

1. Select **Instrument ▶ Device Editor** (Figure 7).



Figure 7 Biomek™ Software Device Editor.

2. From the **Device** menu, select **PTC-200 Left** (Figure 8).

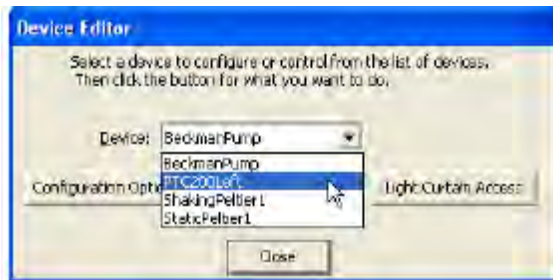


Figure 8 Device drop-down menu.

3. Click **Action Commands**.
4. Select the following (Figure 9):
 - a. In the **Actions** box, select **Close**.
 - b. In the **Open/Close** box, select **Without plate**.

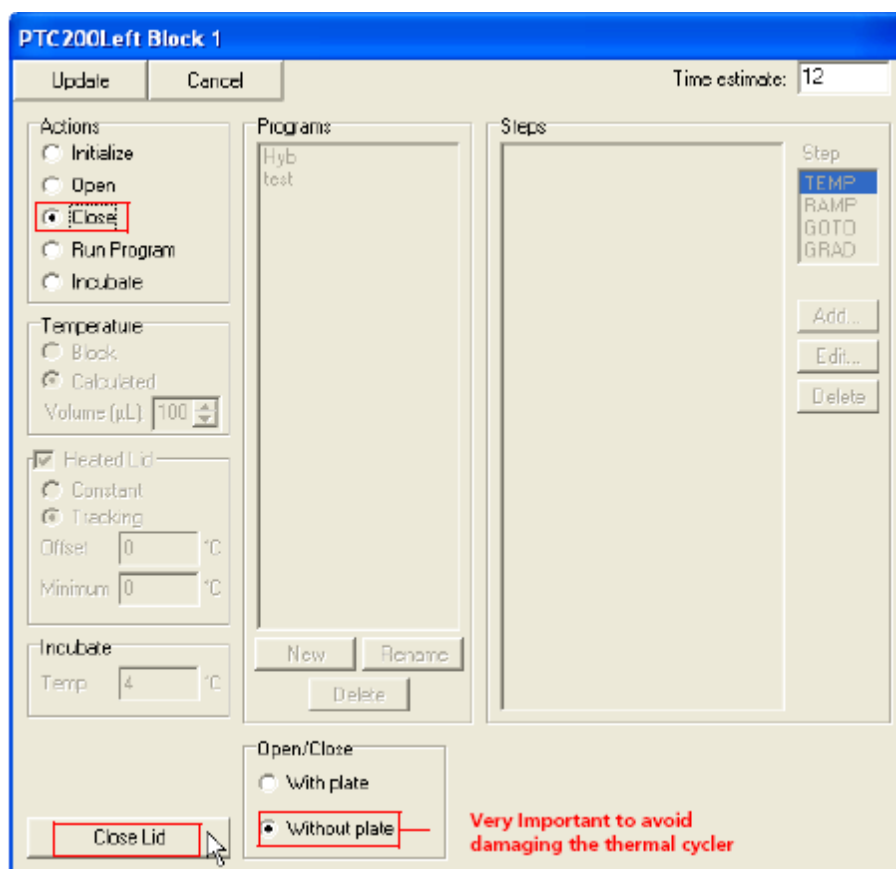


Figure 9 Closing the DNA Engine™ PTC-200 Thermal Cycler lid.

IMPORTANT! It is critical that you select **Without plate** in the **Open/Close** box. If a plate is present, remove it before closing the lid.

5. Click **Close Lid**.

A **Status** box is displayed while the lid is being closed.



6. Click **OK** when the Command executed information box is displayed (Figure 4.5).

7. Click **Cancel**; then click **Close**.

Home all axes

This procedure homes all axes and primes the fluidics lines.

1. Open **Instrument ► Home All Axes**.
2. Ensure that all conditions in the warning prompt Figure 10 (image 1) are met, then click **OK**.



Figure 10 Homing all axes.

A message appears, instructing you to stop and wait during instrument homing (image 2).

3. When:

- a. The warning prompt in Figure 11 (image A) is displayed, confirm that no tips are loaded in the Span-8 Pod, and click **OK**.
 The lines for the Span-8 tips are primed and the next prompt shown in Figure 11 (image B) is displayed.

- b. The intake (syringes and tubing) for the Span-8 tips is clear of bubbles, click **OK**.

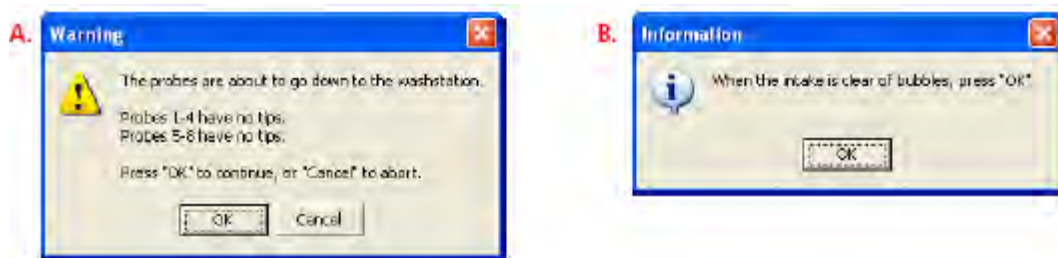


Figure 11 Prompts displayed for priming the Span-8 Pod fluidic lines.

The Biomek™ FX^P TPE System is ready for use.

Related Biomek™ FX^P Target Prep Express Automated Workstation documentation

User manuals are installed with the Biomek™ Software. Click **Start ▶ All Programs ▶ Beckman Coulter ▶ Manuals** to access the manuals.

See the following publications for troubleshooting the Biomek™ instrument.

- *Biomek™ Liquid Handler User's Manual*
- *Biomek™ Software User's Manual*

Breaking the light curtain while running the assay

For your safety, the Biomek™ FX^P Target Prep Express Automated Workstation is equipped with a light curtain (Figure 12) that senses when an object (such as a hand or an arm) enters the space surrounding the deck. When the curtain is broken, the Biomek™ FX^P Target Prep Express Automated Workstation immediately halts all movement on the deck until you click **OK** to resume the activity that was taking place, or abort the activity. Incubation timers are not interrupted.

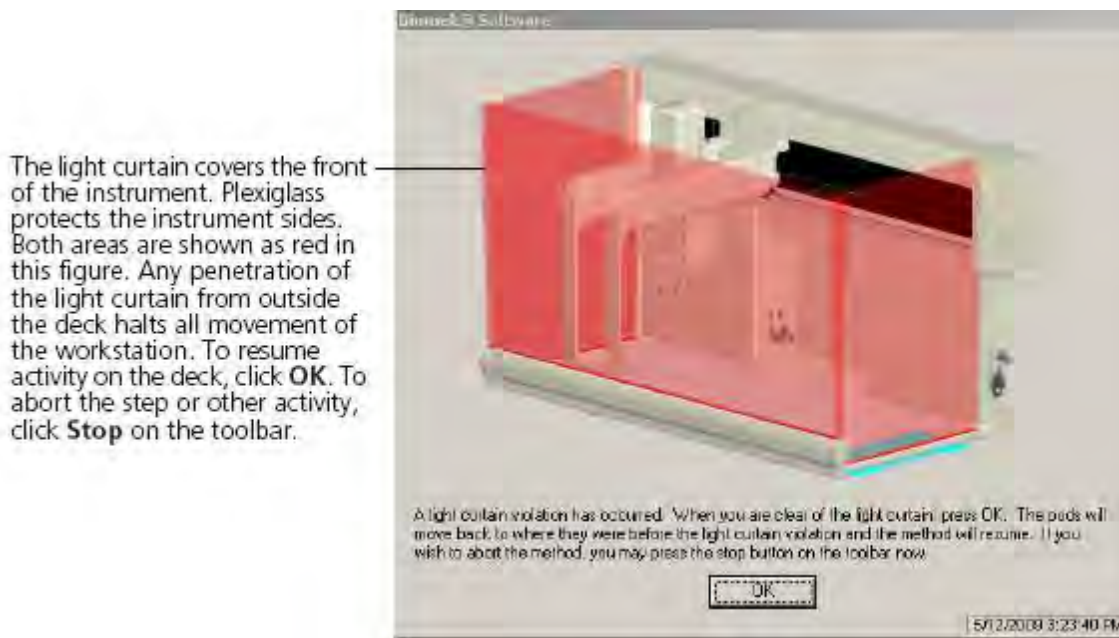


Figure 12 Prompt when the light curtain is broken during the process homing all axes.

Prepare the samples and reagents

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

Thaw and prepare the reagents

IMPORTANT! Before loading the tubes, mix buffer solutions by vortexing. If a precipitant is present in the buffer, dissolve by placing in a heat block at 40°C, vortex, and return to the heat block until dissolved. Mix enzyme solutions by gently flicking the reagent tube a few times. Briefly centrifuge ALL tubes before inserting them into the reagent cold block. Inspect for bubbles. Wipe the bottom of the reagent cold block before placing it on the deck.

1. Thaw the following reagents from the WT Expression Kit on the bench top at room temperature.
 - First Strand Buffer
 - Second Strand Buffer
 - IVT Buffer
2. Vortex and centrifuge the buffers, then place on ice or in the reagent cold block.
3. Gently flick the bottom of each enzyme tube, then centrifuge the tubes and place them on ice or in the reagent cold block with the buffers.

Start the Biomek™ Software

1. If necessary, start the Biomek™ Software by clicking the Biomek™ FX^P Software icon  on the desktop. Alternately, select **Start ▶ All Programs ▶ Beckman Coulter™ ▶ Biomek™ Software** from the menu bar
2. Select the **WT Target Prep** project.
 - a. Select **Project ▶ Open Project** on the menu bar.

- b. In the dialog box that appears (Figure 13), select **WT Target Prep**, then click **OK**.

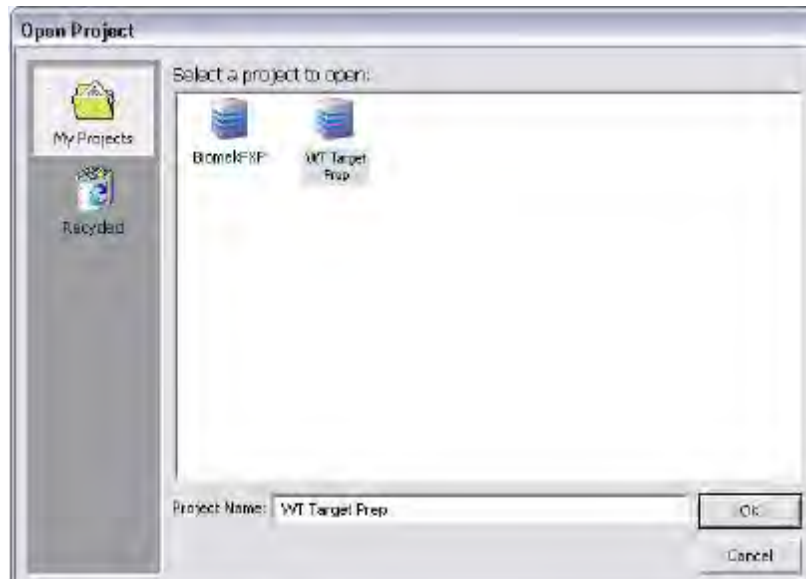


Figure 13 Select the WT Target Prep project.

3. Open the **WT Target Prep** method.
- a. Click **Open** . Alternately, select **File** ► **Open** from the menu bar. The **Open Method** dialog box appears (Figure 14).

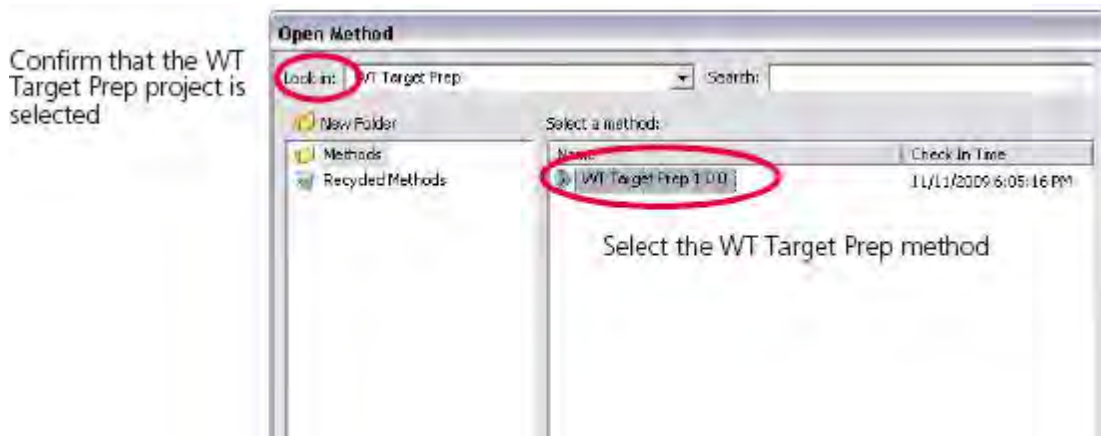


Figure 14 Open Method dialog box.

- b. Confirm that the **WT Target Prep** project is selected in the **Look in** list.
4. Select the **WT Target Prep** method, then click **OK**.
The **Method View** is populated with information (Figure 15).

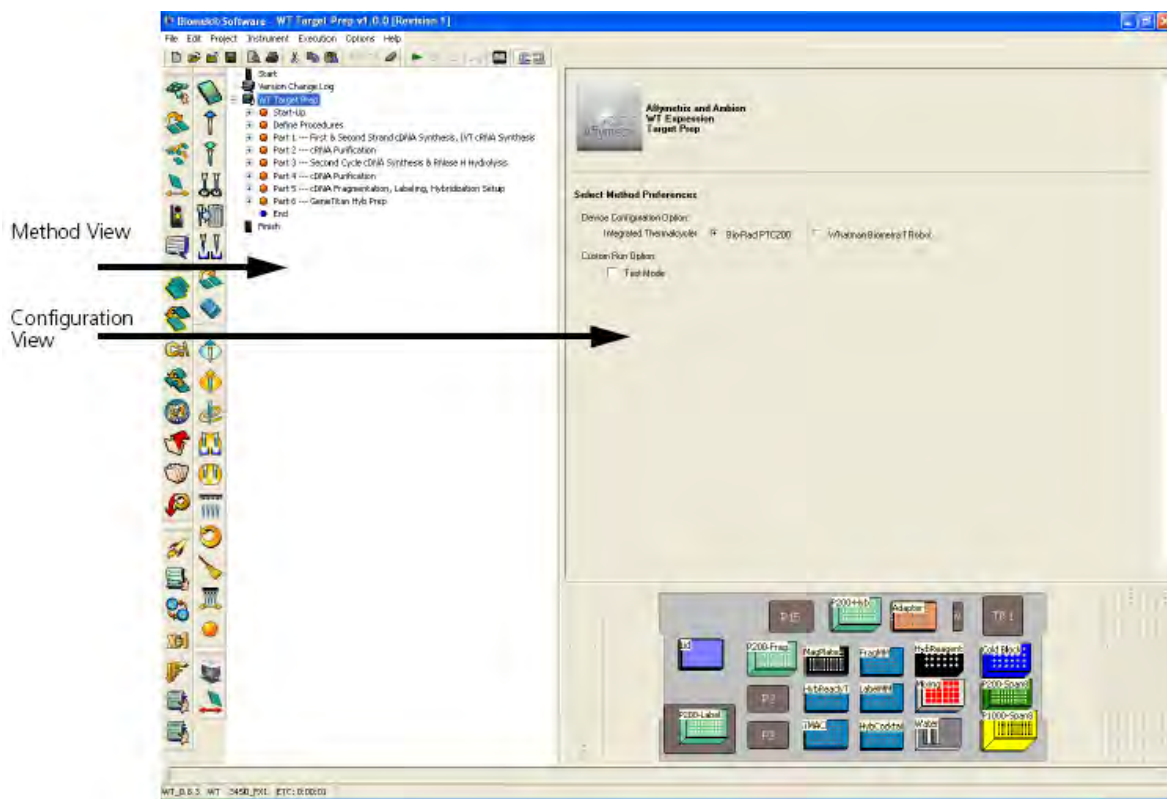


Figure 15 WT method loaded.

5. In the **Method View** directory tree, click **WT Target Prep**.
6. In the **Configuration View** area, under **Device Configuration Option**, confirm that the correct integrated thermal cycler device is selected (DNA Engine™ PTC-200 Thermal Cycler or Biometra™ TRobot 96 Thermocycler).

Note: Test Mode: The **Test Mode Custom Run Option** should be used only for diagnostics and not for live runs. DO NOT select Test Mode because it will disable the timers.

7. Click **Run** .
- The **Run Setting Options** are displayed (Figure 16).

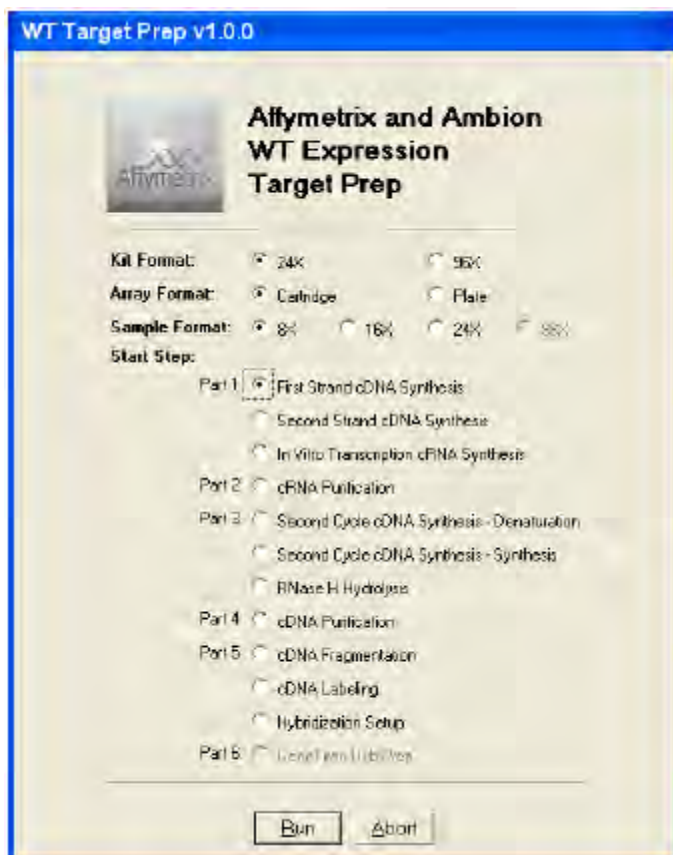


Figure 16 Run settings options.

Note: After clicking **Run**, method validation may take 30 to 60 seconds. Please be patient. Do not click **Run** again.

8. Select the kit format, array format, sample format, and start step:
 - **Kit Format:** Select the format (**24K** or **96K**) for the WT Expression Kit that you are using.
 - **Array Format:** Select **Cartridge** or **Plate**.
 - **Sample Format:** Select the sample format: **8x**, **16x**, **24x**, or **96x**.
 - **Start Step:** Choose the starting step for the run. By default, the method starts at the beginning of Part 1 and proceeds through all subsequent steps. To choose a particular starting step, click the option next to the step.
9. When ready to continue, click **Run**.
 The **Labware & Reagent Setup** window appears (Figure 17).

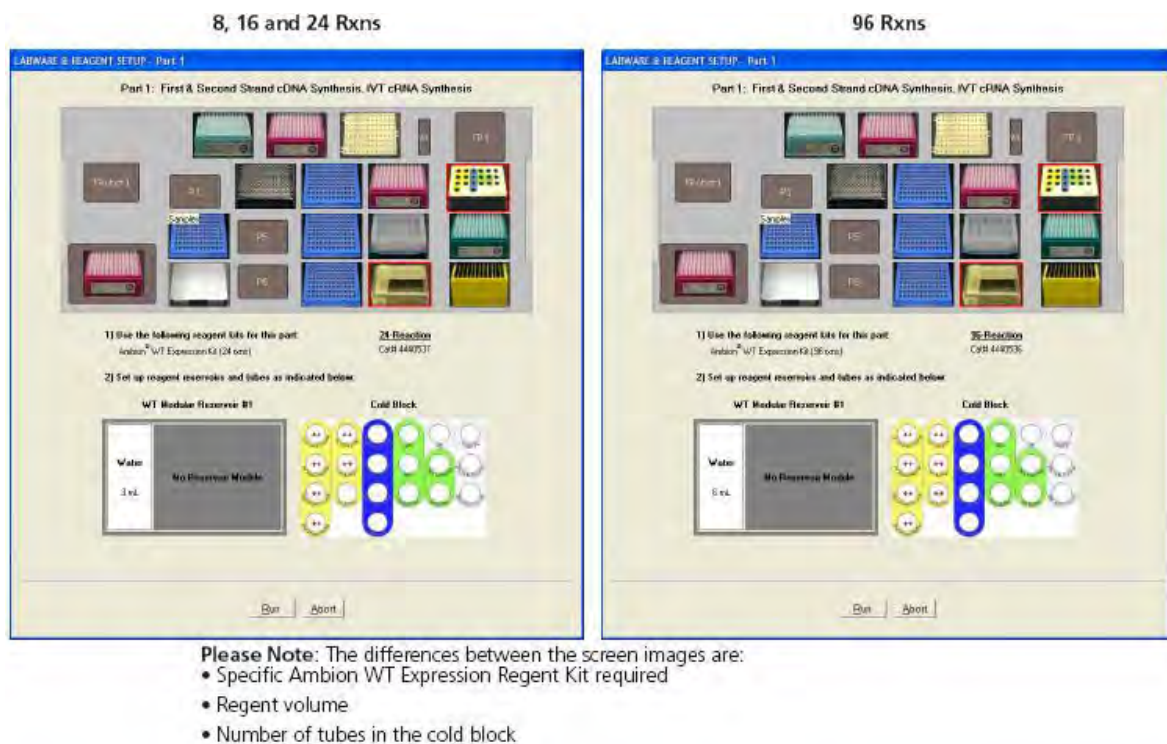


Figure 17 Part 1 labware & reagent setup.

Begin Part 1—First- and Second-strand cDNA Synthesis, IVT cRNA Synthesis

1. After clicking **Run** in the previous task, the **Labware & Reagent Setup** window appears (Figure 17).

IMPORTANT! At position P11 there is an Alpiro™ Plate Cushion under the 24-well mixing plate. See Table 25 for labware setup details.

2. Set up the reagent cold block as shown in Figure 17 or Figure 18 A or B.

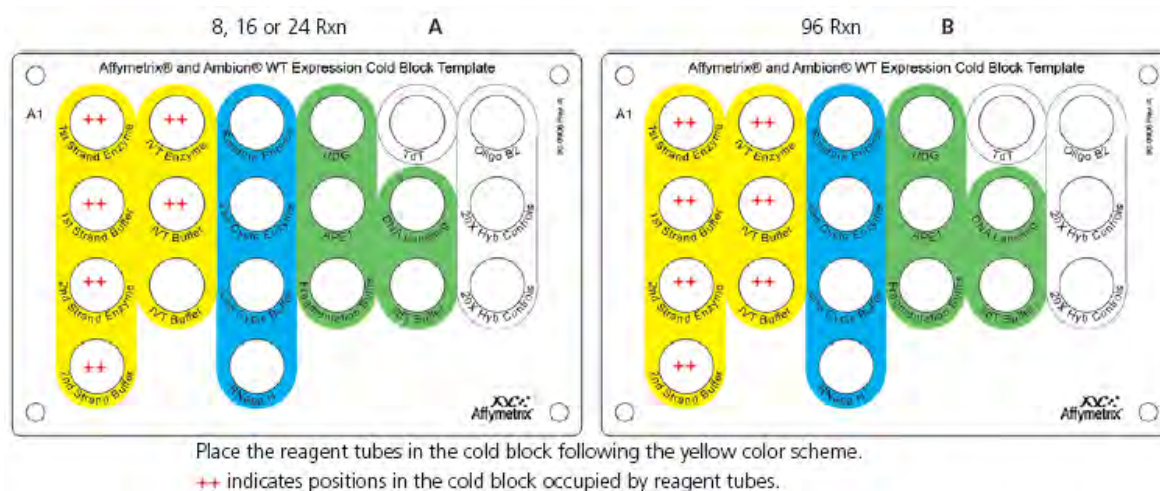


Figure 18 Reagent cold block setup for 8, 16, 24, and 96 reactions for Part 1.

IMPORTANT! All reagents should already be mixed and centrifuged prior to loading in the reagent cold block prechilled to 4°C.

- a. Place the reagent cold block template onto the cooled (4°C) reagent cold block.
 - b. Uncap the First-Strand, Second-Strand, and IVT reagent tubes and insert them into the prechilled cold block following the yellow color code of the template Figure 18.
 - c. Confirm that deck position Pelt_1 is set to 4°C (look at the display of the Watlow controllers below the deck), then place the cold block with the reagents on the deck at position Pelt_1.
3. Place the labware and Modular Reservoir on the deck as directed in the following figures and table:
 - Figure 17—Illustration of the deck layout
 - Figure 19—Location names of empty deck positions

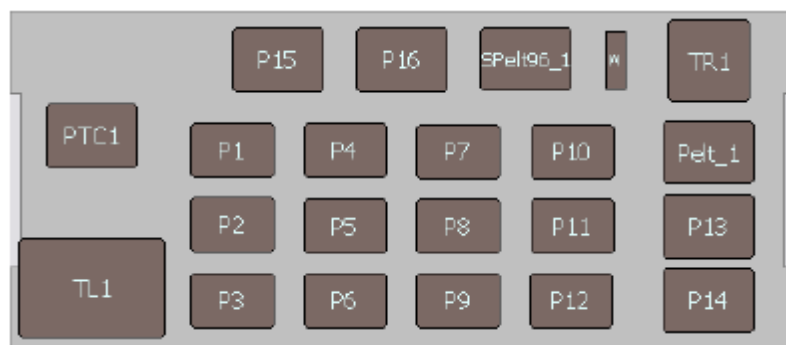


Figure 19 Empty deck positions.

- Table 25— Table detailing the labware and modular reservoir placement for the deck layout

Table 25 Labware and reagent locations on the deck for Part 1.

Position on deck ^[1]	Labware	Use	Reagent or samples			
TL1	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—First Strand				
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Sample	Sample			
P3	Auto-sealing Microplate Lid with Microseal™ P Pad	Lid				
P4	Alpaqua™ Magnetic Plate	Magnetic Plate				
P7	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	First Strand Dist.				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Second Strand Dist.				
P9	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	IVT Dist.				
P10	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—Span8				
P11	Alpillo™ Plate Cushion with 24-well mixing plate	Mixing				
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4
	– Quarter module in position 1 – Positions 2, 3 and 4 are empty – Pour Water into reservoir 1 – 8 reactions = 3 mL – 16 reactions = 3 mL – 24 reactions = 3 mL – 96 reactions = 6 mL		Water	Empty		
Pelt 1	Reagent Cold Block, chilled to 4°C	Cold Block	See Step 2 for detailed setup instructions			
P13	Biomek™ Span P250 Barrier Tips ^[2] (green)	P200—Span8				
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000—Span8				
P15	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Second Strand				
P16	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—IVT				
Spelt96_1	Peltier Adapter (installed on deck)					

^[1] See Figure 19 for empty deck positions.

^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

4. When the deck is ready, click **Run** in the **Labware & Reagent Setup** window (Figure 17).
 You are prompted to purge the Pod2 syringe lines.

Note: Verify that you have removed:

- Adhesive seals, if any.
- Caps from reagent tubes on the cold block.
- Lids from the tip boxes.

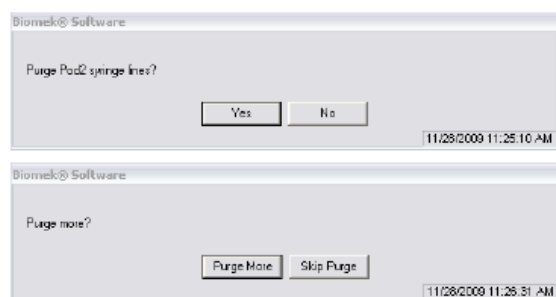


Figure 20 Purge messages.

5. Click **Yes** in the prompt.

If bubbles are still present after purging, click **Purge More** in the next prompt. If no visible bubbles in tubing or syringes, click **Skip Purge**.

The robot proceeds without user intervention with Part 1 through the IVT cRNA synthesis, which is held in the thermal cycler for 16 hours. When Part 1 is complete, the message shown in Figure 21 appears.

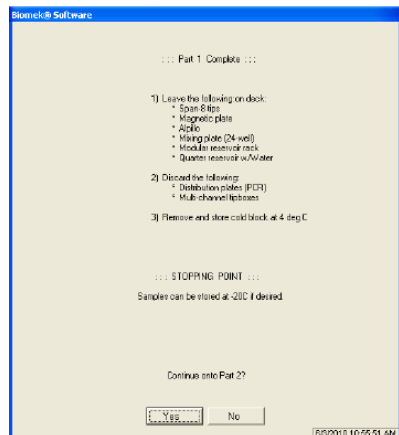


Figure 21 Message at completion of Part 1.

6. Follow the instructions in the message.
 - a. Leave the following on deck:
 - Span-8 tips
 - Magnetic plate
 - Mixing plate (24-well)
 - Modular reservoir frame and quarter reservoir with water
 - b. Discard the distribution plates (PCR) (for locations of the distribution plates see Figure 19) and multichannel tip boxes.

- c. Remove and store the reagent cold block at 4°C.

STOPPING POINT After Part 1 is complete, the samples can be stored at –20°C.

7. To continue on to Part 2, click **Yes**.
8. If you do not want to continue with Part 2, click **No**. When ready to resume, ensure that clean, fresh Span-8 tip boxes are placed on the deck, then click **Run** ► in the window that appears (Figure 22).

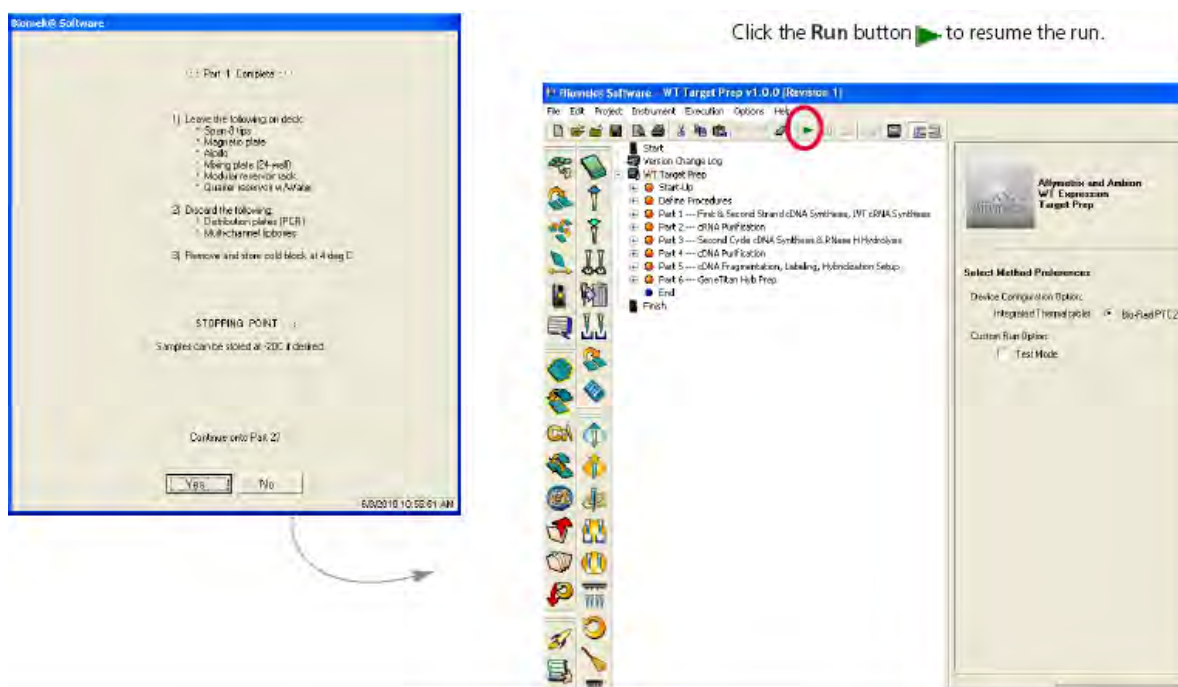


Figure 22 Message at completion of part 1.

Part 2—cRNA Purification

After the IVT synthesis is complete, the cRNA is purified to remove enzymes, salts, and unincorporated nucleotides. At completion, Part 2 yields a Bio-Rad™ Hard-Shell 96 PCR plate with quantified and normalized cRNA.

Approximate time required for Part 2

Part 2—cRNA Purification	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: cRNA purification	63	64	65	83
Normalization	3	3	3	11
Part 2 total time	81 1.35 hours	82 1.37 hours	83 1.38 hours	109 1.82 hours

Reagents and labware required for Part 2

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 26 Part 2 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput, 4440537, 24 reactions^[1] or 4440536, 96 reactions. Components needed: • Nucleic Acid Binding Buffer Concentrate • Nucleic Acid Binding Beads • Nucleic Acid Wash Concentrate • Elution Solution • Nuclease-free Water • Isopropanol • Ethanol, 200 proof

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: All components and reagents, except beads, for Part 2 and Part 4 should be at room temperature. Allow samples to sit at room temperature for at least 10 minutes. The Bead Binding Buffer will precipitate if stored at 4°C. Ensure that the binding buffer and beads are at room temperature prior to starting the procedure.

Table 27 Part 2 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ AP96 – P250 Barrier Tips (aqua) Beckman Coulter™, 717253
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
2	2	2	2	Costar™ U-bottom 96-well, Polystyrene Plate Costar™, 3798, or part of 901646
1	1	1	2	ABgene™ 96 Square Well Storage Plate ABgene™, AB-0932, or part of 901646
1	1	1	2	Greiner Bio-One™ UV-Star™ Plate Greiner Bio-One™, 655801, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2453, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790

Table 27 Part 2 labware requirements. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
2	2	2	2	Quarter Reservoir Divided by Width Beckman Coulter™, 372792
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Perform a prerun check of the Biomek™ FX^P TPE System

Note: The prerun check is not required if you continue on to Part 2 without stopping the method at the end of Part 1.

The following actions are the same as described under “Prepare the Biomek™ FX^P TPE System” on page 43. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek™ FX^P Target Prep Express Automated Workstation.



CAUTION! If using the DNA Engine™ PTC-200 Thermal Cycler, before turning it on confirm that the multichannel head (pod_1) is not positioned over the thermal cycler.

1. Power on the workstation and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek™ Software.
4. If applicable, close the DNA Engine™ PTC-200 Thermal Cycler lid.
5. Home all axes.

Prepare the samples and reagents

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

Gather the cRNA purification reagents

Note: All components and reagents should be at room temperature. Allow samples to sit at room temperature for at least 10 minutes. The Bead Binding Buffer will precipitate if cold. Ensure that the binding buffer and beads are at room temperature prior to starting the procedure.

1. Gather the following reagents and place them on the bench top at room temperature.

- Binding Beads
- Binding Beads Buffer
- Wash Solution

IMPORTANT! Add ethanol to wash solution prior to use. Follow the instructions on the bottle.

- Elution Buffer
- Isopropanol
- Nuclease-free water

2. Vortex and mix the beads thoroughly. Ensure that no beads are clumped together at the bottom of the tube.

Continue the run—Part 2—cRNA Purification

If you are beginning the run at Part 2, click **Run** below the menu bar, then make selections from the **Run Settings Options** window. Then begin Part 2 at step 2.

1. If prompted, place a new box of tips as indicated in the message prompt. When ready to continue click **OK**.
2. The **Labware & Reagent Setup** window for Part 2 appears (Figure 23).

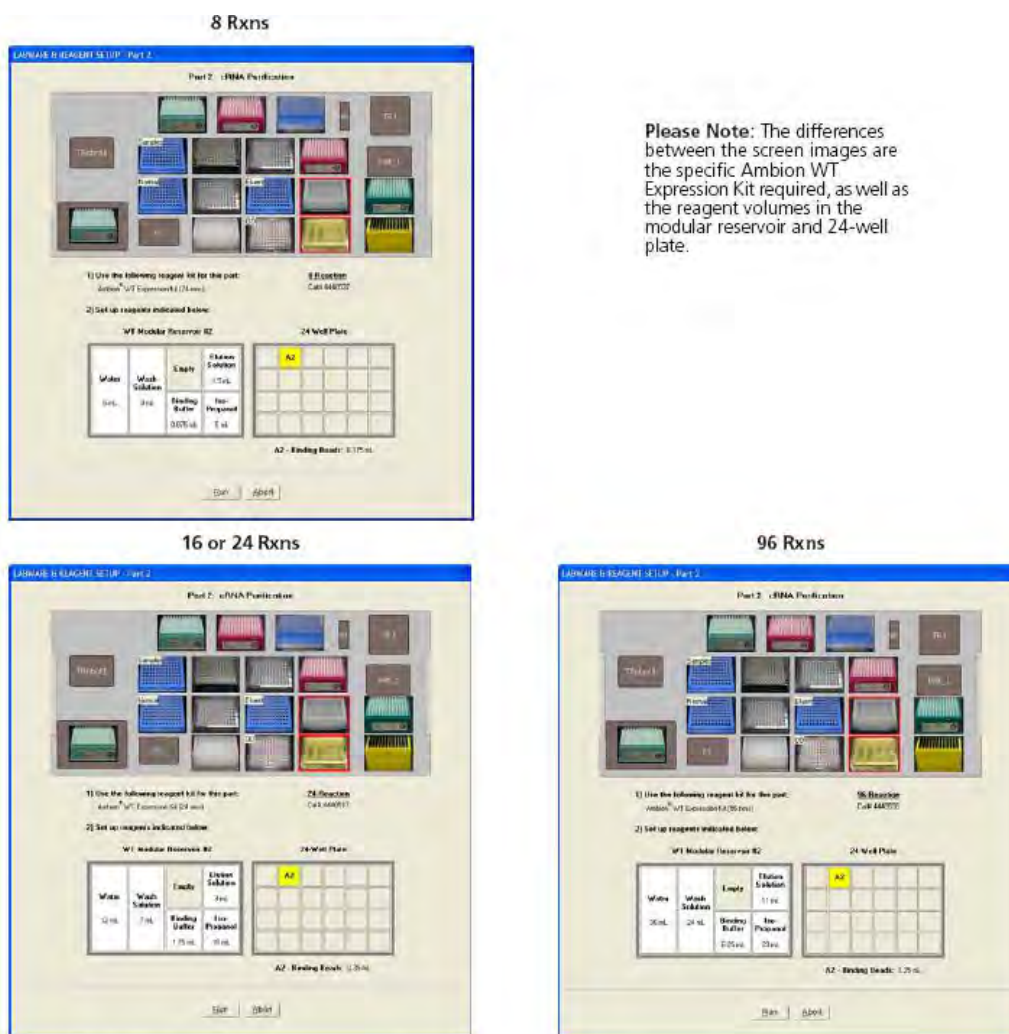


Figure 23 Part 2 labware & reagent setup.

IMPORTANT! Use nonstick RNase-free microfuge tubes (not included) to prepare all of the dilutions.

Note: Keep the reagent cold block in the refrigerator when not in use.

3. Set up the 24-well plate and modular reservoir, then place the labware on the deck as directed in the following figures and table:

- Figure 23—Illustration of the deck layout
- Figure 24—Pipette the correct reagent volumes in the 24-well plate

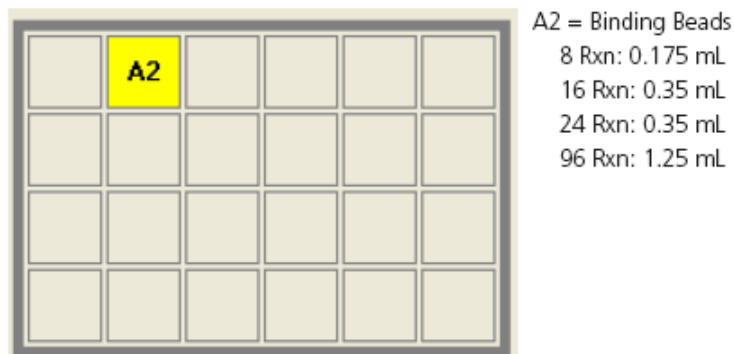


Figure 24 24-well plate for Part 2 deck setup.

- Figure 25—Location names of empty deck positions

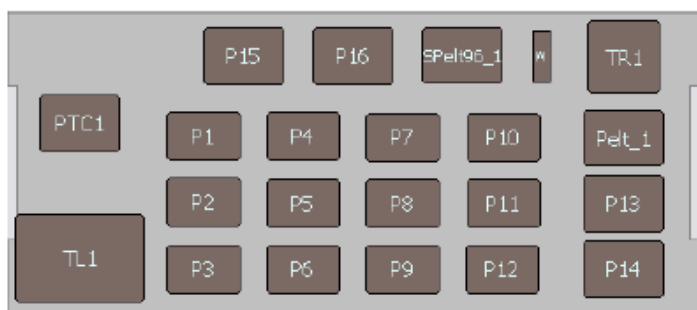


Figure 25 Empty deck positions.

- Table 28—Pipette the correct reagent volumes in the modular reservoir. This table describes the labware and modular reservoir placement for the deck layout.

Table 28 Labware and reagent locations on the deck for Part 2.

Position on deck ^[1]	Labware	Use	Reagent or samples
TL1	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Beads	
P1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Sample	Sample
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Normal	
P4	Alpaqua™ Magnetic Plate	Bead Magnet	
P5	Costar™ Polystyrene 96-well, U-bottom Plate	Bead Binding	

Table 28 Labware and reagent locations on the deck for Part 2. *(continued)*

Position on deck ^[1]	Labware	Use	Reagent or samples			
P6	ABgene™ 96 Square Well Storage Plate	IPA Waste				
P7	Costar™ Polystyrene 96-well, U-bottom Plate	Wash Solution				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Eluent				
P9	Greiner Bio-One™ UV-Star™ Plate	OD				
P10	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—Span8				
P11	Alpillo™ Plate Cushion with 24-well mixing plate	Mixing	See Figure 24 for setup instructions			
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4
			Water	Wash Solution	Empty	Elution Solution

Table 28 Labware and reagent locations on the deck for Part 2. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples			
P12	- Quarter module reservoir in position 1 and 2 - Quarter module reservoir divided by width in positions 3 and 4 - Pipette Water into reservoir 1 8 reactions = 6 mL 16 reactions = 12 mL 24 reactions = 12 mL 96 reactions = 36 mL - Pipette Wash Solution into reservoir 2 8 reactions = 3 mL 16 reactions = 7 mL 24 reactions = 7 mL 96 reactions = 24 mL - Pipette Binding Buffer into the bottom half of reservoir 3 8 reactions = 0.875 mL 16 reactions = 1.75 mL 24 reactions = 1.75 mL 96 reactions = 6.25 mL - Pipette Elution Solution into the top half of reservoir 4 8 reactions = 1.5 mL 16 reactions = 3 mL 24 reactions = 3 mL 96 reactions = 11 mL - Pipette Isopropanol into the bottom half of reservoir 4 8 reactions = 5 mL 16 reactions = 10 mL 24 reactions = 10 mL 96 reactions = 20 mL		Water	Wash Solution	Binding Buffer	Isopropanol
P13	Biomek™ Span P250 Barrier Tips ^[2] (green)	P200—Span8				
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000—Span8				
P15	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Elution				

Table 28 Labware and reagent locations on the deck for Part 2. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples
p16	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—OD	
Spelt96_1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Elution Buffer	

^[1] See Figure 25 for empty deck positions.

^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

Note: If the run was stopped at the end of Part 1, manually place the sample plate at P1 without a lid and ensure that you have placed clean fresh Span-8 tip boxes on the deck before resuming Part 2.

- When the deck is ready, click **Run** in Figure 23.
- In the prompt that appears (Figure 26), click **Yes** to begin purging the Pod2 syringe lines. If bubbles are still present after purging, click **Purge More** in the next prompt (Figure 26). If no further purging is required, click **Skip Purge**.

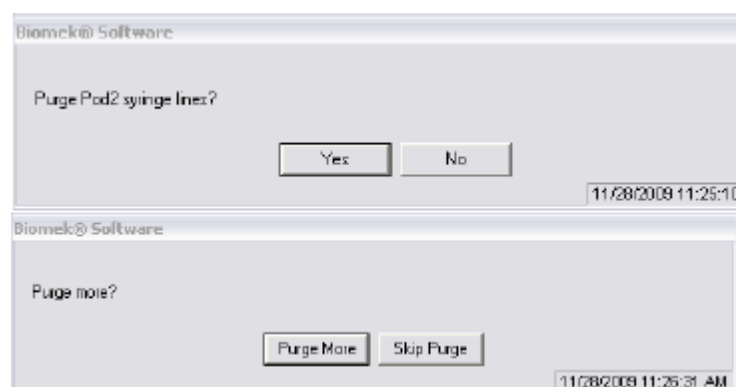


Figure 26 Prompts to purge Pod2 syringe lines.

The run proceeds. The Biomek™ FX^P TPE System begins the cRNA Purification method and proceeds through elution. After a blank OD plate is prepared (contains diluent only), a message appears prompting you to read the plate (Figure 27).



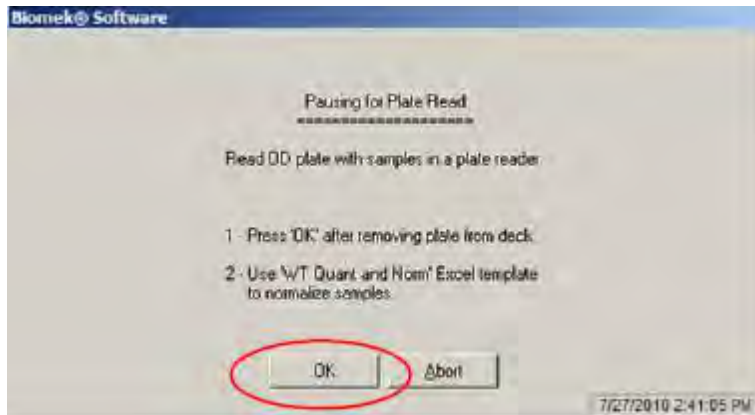
Figure 27 Prompt to read OD plate background.

6. Remove the OD plate from position P9 on the deck.
7. Read background OD in a plate reader at 260 nm.
8. Export or save background OD data for later use.
9. After the plate is read, remove it from the plate reader and place it on deck position P9. Click **OK** in the prompt (Figure 28) to proceed with sample transfer into the OD plate.



Figure 28 Prompt to read OD plate background.

10. Click **OK** after removing OD plate from P9 when prompted again to read OD plate.



Note: The WT Quant and Norm.xls template (Figure 29) opens in the background waiting for OD data.

11. Read sample OD in plate reader at 260 nm.
12. Export or save sample OD data.
13. Return to the WT Quant and Norm.xls template (Figure 29).

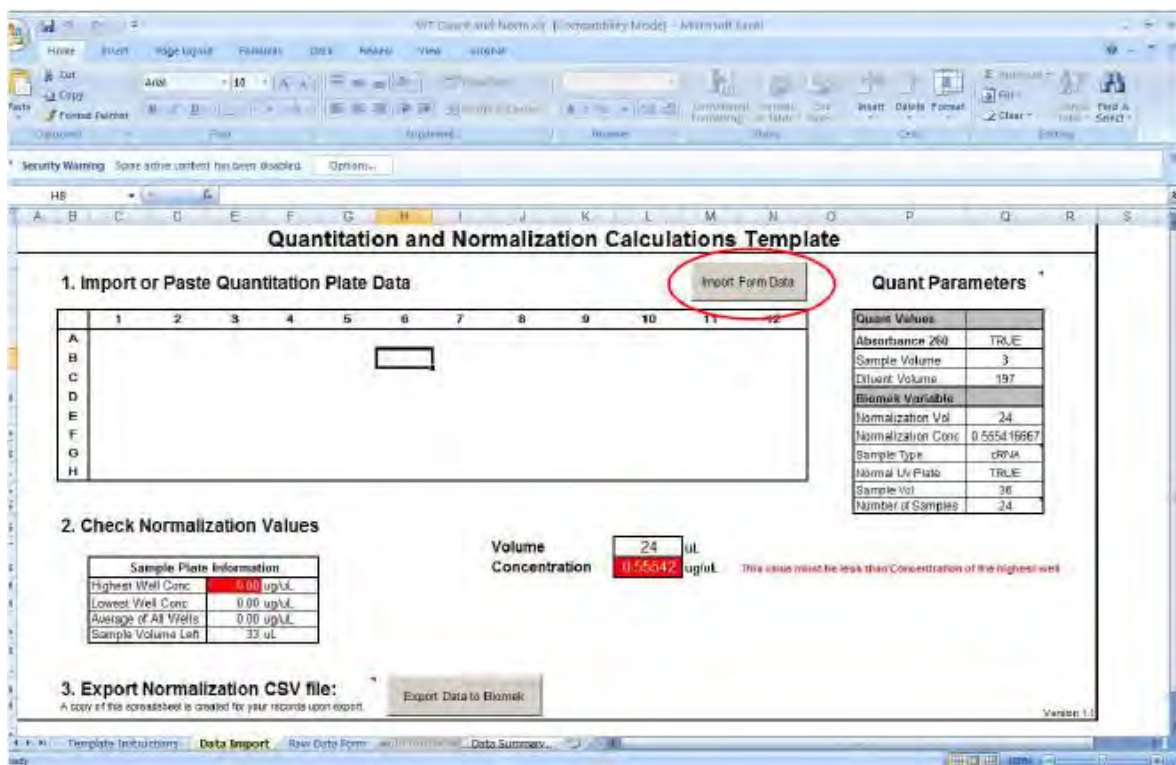


Figure 29 WT quant and norm.xls template.

14. In the Quantitation and Normalization Calculations template, click **Import Form Data**. The Raw Data Form worksheet is shown (Figure 30).

Figure 30 Raw data form worksheet.

15. Copy and paste background OD data saved in step 8 into Section 1 of the Raw Data Form (Figure 31).

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0638	0.0637	0.065	0.0629	0.0612	0.0626	0.0655	0.0626	0.0656	0.0615	0.0654	0.0653
B	0.0633	0.0664	0.0671	0.0611	0.0663	0.0656	0.0656	0.0652	0.0653	0.0647	0.0647	0.0657
C	0.0637	0.0658	0.0671	0.0646	0.0642	0.0626	0.0639	0.0655	0.0617	0.0634	0.0634	0.0650
D	0.0645	0.0648	0.0671	0.064	0.0643	0.0642	0.0652	0.0652	0.064	0.0631	0.0634	0.0649
E	0.0628	0.065	0.0671	0.0633	0.0642	0.0672	0.0673	0.0671	0.0678	0.0678	0.0673	0.0678
F	0.0668	0.0668	0.0668	0.0667	0.0673	0.0666	0.0672	0.0671	0.0668	0.0668	0.0661	0.0677
G	0.0644	0.0654	0.0628	0.0653	0.0636	0.0676	0.0676	0.0656	0.0653	0.0638	0.0648	0.0648
H	0.0637	0.067	0.0657	0.0629	0.0674	0.067	0.0657	0.0656	0.0642	0.0639	0.0651	0.0643

Section 1:
Paste Raw
Background Data
here

Figure 31 Paste raw background data.

16. Copy and paste sample OD data saved in step 12 into Section 2 of the Raw Data Form (Figure 32).

The screenshot shows a Microsoft Excel spreadsheet titled 'RAW DATA FORM'. It contains two main data entry sections:

- 1. Paste Raw Background Data:** A table with 12 columns (1-12) and 8 rows (A-H). It contains numerical data for background readings.
- 2. Paste Raw Sample Data:** A table with 12 columns (1-12) and 8 rows (A-H). It is currently empty, intended for pasting sample OD data.

Below these sections is a table labeled 'Adjusted Sample Data' with a button 'Copy Adjusted Data to Template' circled in red. A red arrow points from the text 'Section 2: Paste Raw Sample Data here' to the empty table in section 2. Another red arrow points from the text 'Automatically Calculated' to the 'Adjusted Sample Data' table.

Figure 32 Paste raw sample data.

17. Click **Copy Adjusted Data to Template** to copy the adjusted OD values to the template

The screenshot shows a Microsoft Excel spreadsheet titled 'Quantitation and Normalization Calculations Template'. It includes several sections:

- 1. Import or Paste Quantitation Plate Data:** A table with 12 columns (1-12) and 8 rows (A-H) containing numerical data.
- 2. Check Normalization Values:** A section with 'Sample Plate Information' (Highest Well Conc: 0.24 ug/uL, Lowest Well Conc: 0.17 ug/uL, Average of All Wells: 0.19 ug/uL, Sample Volume Left: 33 uL) and 'Volume Concentration' (24 uL, 0.55542 ug/uL).
- 3. Export Normalization CSV file:** A section with a button 'Export Data to Biomek' circled in red.
- Quant Parameters:** A table on the right with parameters like Absorbance 260 (TRUE), Sample Volume (3), Diluent Volume (197), Biomek Variable, Normalization Vol (24), Normalization Conc (0.555416667), Sample Type (cRNA), Normal UV Plate (TRUE), Sample Vol (36), and Number of Samples (24).

A red arrow points from the text 'Click Copy Adjusted Data to Template' to the 'Copy Adjusted Data to Template' button in the previous figure.

18. Check the yield by clicking the **Data Summary** tab. The next step requires a minimum of 13.3 μg in 24 μL volume.



CAUTION! Normalizing below the required minimum sample concentration at this point is not recommended and may yield undesired results. The normalization process can proceed, if desired.

- Samples that are below the minimum of 5.5 μg are carried through the remainder of the procedure but are not properly normalized. Hybridization results from improperly normalized samples may not be valid.
- Low OD readings may be due to bubbles in sample wells. Check the plate and inspect the UV plate for bubbles. If bubbles are present, carefully remove them using a clean pipette tip.

19. In the **Data Import** tab, click **Export Data to Biomek** in the Quantitation and Normalization Calculations Template.

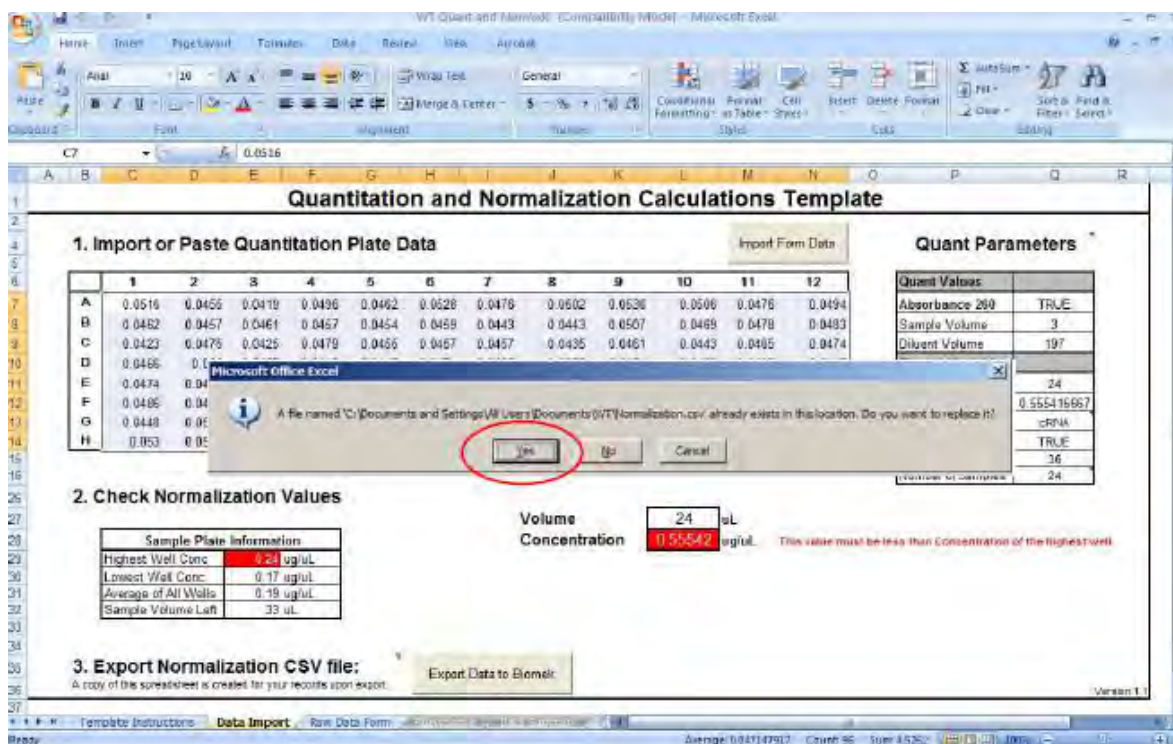


Figure 33 Quantitation and Normalization Calculations template.

If any of the samples do not meet the minimum concentration a warning message appears (Figure 34). Click **Yes** in the prompt that appears (Figure 33).

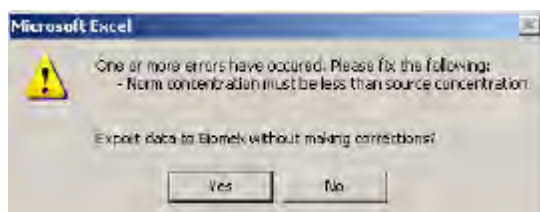


Figure 34 Warning message.

The next dialog box that appears (Figure 35) enables you to save a copy of the background-corrected data and the parameters used to determine sample normalization. Saving a backup copy is optional.

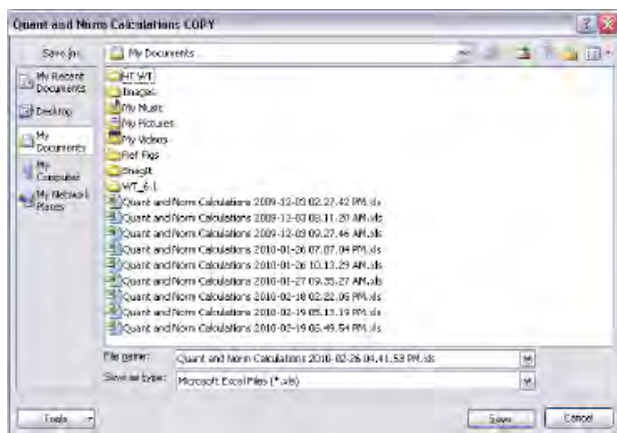


Figure 35 Prompt to save a copy of the OD data and normalization parameters.

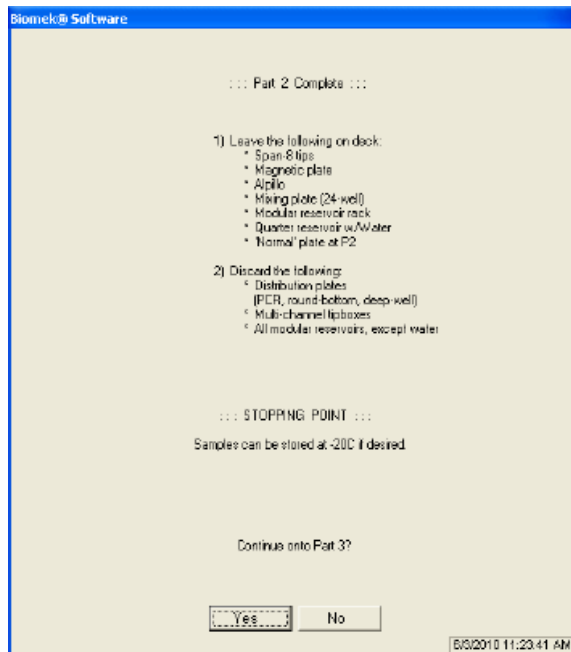
20. To save a backup copy of the data and normalization parameters:
 - a. Confirm the default folder or select a new folder.
 - b. Confirm the default file name or enter a new file name.

21. Click **Save**.

The sample volumes and concentrations are normalized using elution buffer as the diluent. The Quantitation and Normalizations Template closes.

22. Remove the OD plate from plate reader and discard.

23. When Part 2 is complete, the following message appears.



STOPPING POINT After Part 2 is complete, the samples can be stored at -20°C.

24. Follow the instructions in the message.

a. Leave the following on deck:

- Span-8 tips
- Magnetic plate
- Mixing plate (24-well)
- Quarter modular reservoir with water
- "Normal" plate at deck position P2

b. Discard the following:

- Distribution plate (PCR, round-bottom, deep-well)
- Multichannel tip boxes
- All modular reservoirs, except water

25. To continue on to Part 3, click **Yes** (see "Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis" on page 73).

26. If you do not want to continue with Part 3, click **No**. When ready to resume ensure that clean, fresh Span-8 tip boxes are placed on the deck, then click **Run**  in the window that appears (Figure 36).

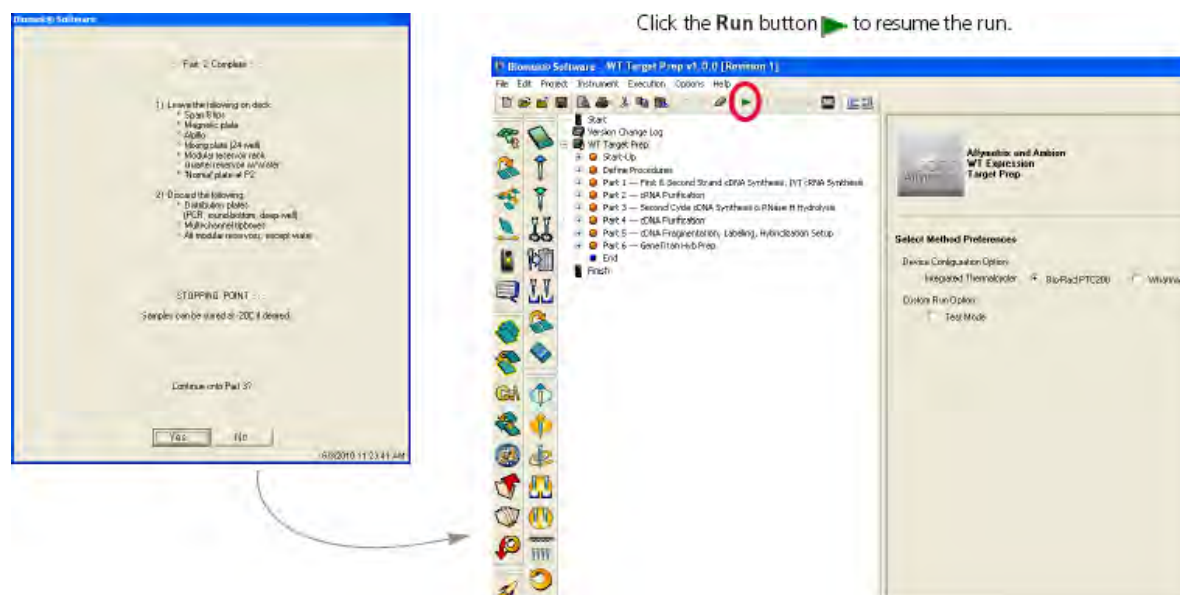


Figure 36 Stopping/resuming the run.

Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis

Sense-strand cDNA is synthesized by the reverse transcription of cRNA. At completion, Part 3 yields a Bio-Rad™ Hard-Shell™ 96-Well PCR Plate with hydrolyzed ss-cDNA samples.

Approximate time required for Part 3

Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	10	10	10	10
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: second-cycle denaturation	4	5	5	10
Thermal cycler: denaturation method	12	12	12	12
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: second-cycle synthesis	5	5	5	15
Thermal cycler: synthesis method	110	110	110	110

(continued)

Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: RNase H hydrolysis	4	5	5	11
Thermal cycler: RNase H method	50	50	50	50
Part 3 total time	195 3.25 hours	197 3.28 hours	197 3.28 hours	218 3.63 hours

Reagents and labware required for Part 3

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 29 Part 3 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 , 24 reactions ^[1] or 4440536 , 96 reactions Components needed: • Random Primers • Second-Cycle Enzyme Mix • Second-Cycle Buffer Mix • RNase H • Nuclease-free Water

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Table 30 Part 3 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
0 tips	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	0 tips	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™ HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
1	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Perform a prerun check of the Biomek™ FX^P TPE System

Note: The prerun check is not required if you continue on to Part 3 without stopping the method at the end of Part 2.

The following actions are the same as described under “Prepare the Biomek™ FX^P TPE System” on page 43. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek™ FX^P Target Prep Express Automated Workstation.

1. Power on the workstation and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek™ Software.
4. If applicable, close the DNA Engine™ PTC-200 Thermal Cycler lid.
5. Home all axes.

Prepare the samples and reagents

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

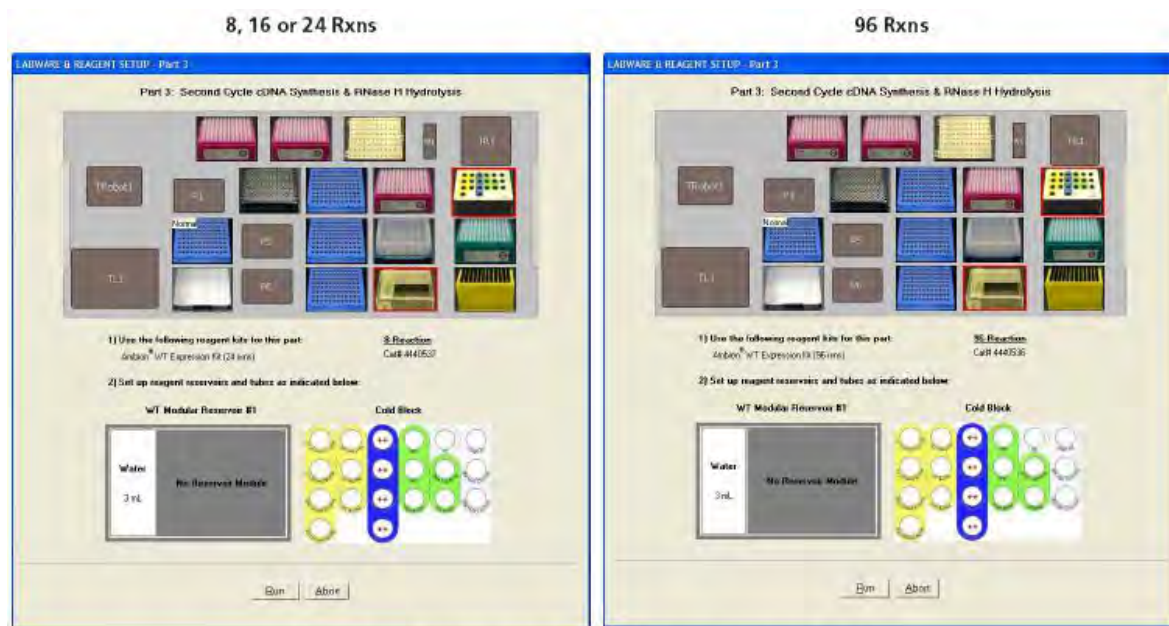
Thaw and prepare the reagents

1. Thaw the following reagents from the WT Expression Kit on the bench top at room temperature.
 - Random Primer
 - Second-Cycle Buffer Mix
2. Vortex and centrifuge the reagents, then place on ice.
3. Gently flick the bottom of each enzyme tube, then centrifuge the tubes and place them on ice with the buffers.

Continue the run—Part 3—Second-cycle cDNA Denaturation, cDNA Synthesis, and RNase H Hydrolysis

If you are beginning the run at Part 3, click **Run** below the menu bar, then make selections from the **Run Settings Options** window. Then begin Part 3 at step 2.

1. If prompted, place a new box of tips as indicated in the message prompt. When ready to continue click **OK**.
2. The **Labware & Reagent Setup** window appears (Figure 37).



Please Note: The difference between the screen images is the specific Ambion WT Expression Kit required.

Figure 37 Part 3 labware and reagent setup.

IMPORTANT! At position P11 there is an Alpipto™ Plate Cushion under the 24-well mixing plate. See Table 31 for labware setup details.

3. Set up the reagent cold block as shown in Figure 37 or Figure 38 A or B.

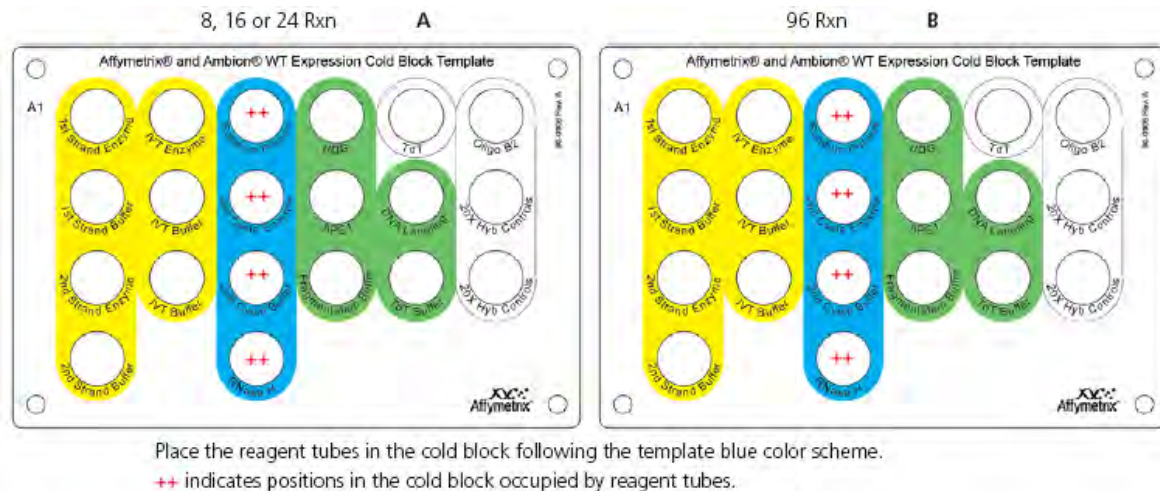


Figure 38 Reagent cold block setup for 8, 16, 24, and 96 reactions for Part 3.

- Place the reagent cold block template onto the cooled (4°C) cold block.
- Uncap the Random Primers, Second-Cycle Enzyme Mix, Second-Cycle Buffer Mix, and RNase H reagent tubes and insert them into the cooled reagent cold block following the blue color code of the template (Figure 38). Place the reagent cold block on the deck at position Pelt_1.

IMPORTANT! Before loading the tubes, mix buffer solutions by vortexing. If a precipitant is present in the buffer, dissolve by placing in a heat block at 40°C, vortex, then return to the heat block until dissolved. Mix enzyme solutions by gently flicking the reagent tube a few times. Briefly centrifuge ALL tubes before inserting them into the reagent cold block. Inspect for bubbles. Wipe the bottom of the reagent cold block before placing it on the deck.

4. Place the labware and modular reservoir on the deck as directed in the following figures and table:
- Figure 37—Illustration of the deck layout
 - Figure 39— Location names of empty deck positions

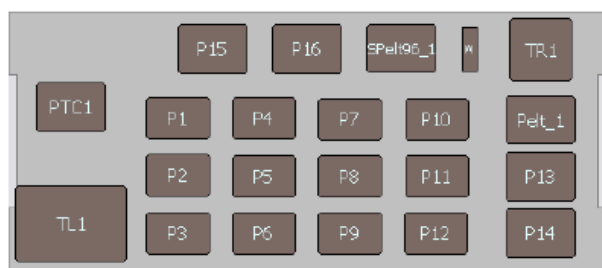


Figure 39 Empty deck positions.

- Table 31—Table detailing the labware and Modular Reservoir placement for the deck layout

Table 31 Labware and reagent locations on the deck for Part 3.

Position on deck ^[1]	Labware	Use	Reagent or samples			
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Normal	Normalized cRNA from Part 2			
P3	Auto-sealing Microplate Lid with Microseal™ P Pad	Lid				
P4	Alpaqua™ Magnetic Plate	Magnetic Plate				
P7	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Random Primers				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Second Cycle MM				
P9	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	RNase H				
P10	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—Span8				
P11	Alpillo™ Plate Cushion with 24 well mixing plate	Mixing				
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4
	– Quarter module in position 1 – Positions 2, 3 and 4 are empty – Pour Water into reservoir 1 – 8 reactions = 3 mL – 16 reactions = 3 mL – 24 reactions = 3 mL – 96 reactions = 6 mL		Water			
Pelt_1	Reagent Cold Block, chilled to 4°C	Cold Block	See Step 3 for detailed setup instructions			
P13	Biomek™ Span P250 Barrier Tips ^[2] (green)	P200—Span8				
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000—Span8				
P15	Biomek™ AP96—P250 Barrier Tips (aqua)	P50—Sample				
P16	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—RNase H				
Spelt96_1	Peltier Adapter (installed on deck)					

^[1] See Figure 39 for empty deck positions.

^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

5. When the deck is ready, click **Run.**

You are prompted to purge the Pod2 syringe lines.

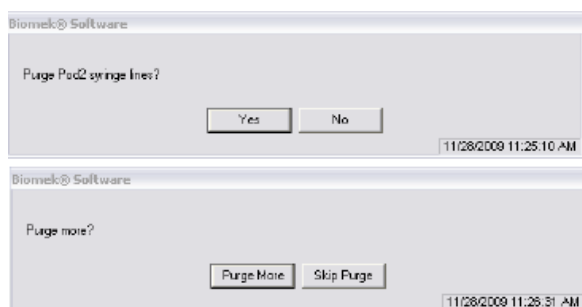


Figure 40 Purge messages.

6. If bubbles are present in the Pod2 syringe lines, click **Yes** to begin purging.
7. If necessary, click **Purge More** in the next prompt (Figure 26). If no further purging is required, click **Skip Purge**.

No user intervention is required in Part 3 if you are using an integrated thermal cycler. The run proceeds without further user intervention. When Part 3 is complete, the following message appears.

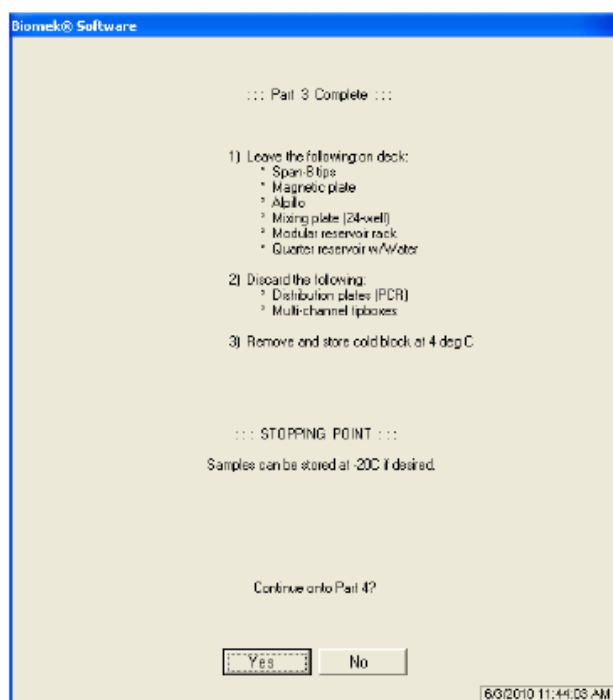


Figure 41 Message at completion of Part 3.

STOPPING POINT After Part 3 is complete, the samples can be stored at -20°C .

8. Follow the instructions in the message.
 - a. Leave the following on deck:
 - Span-8 tips
 - Magnetic plate
 - Mixing plate (24-well)
 - Modular reservoir frame
 - b. Quarter reservoir with water
 - c. Discard the distribution plates (PCR) and multichannel tip boxes
 - d. Remove and store the reagent cold block at 4°C.
9. To continue on to Part 4, click **Yes**.
10. If you do not want to continue the run, click **No**. When ready to resume, ensure that clean, fresh Span-8 tip boxes are placed on the deck and click **Run** ► in the window that appears (Figure 36).

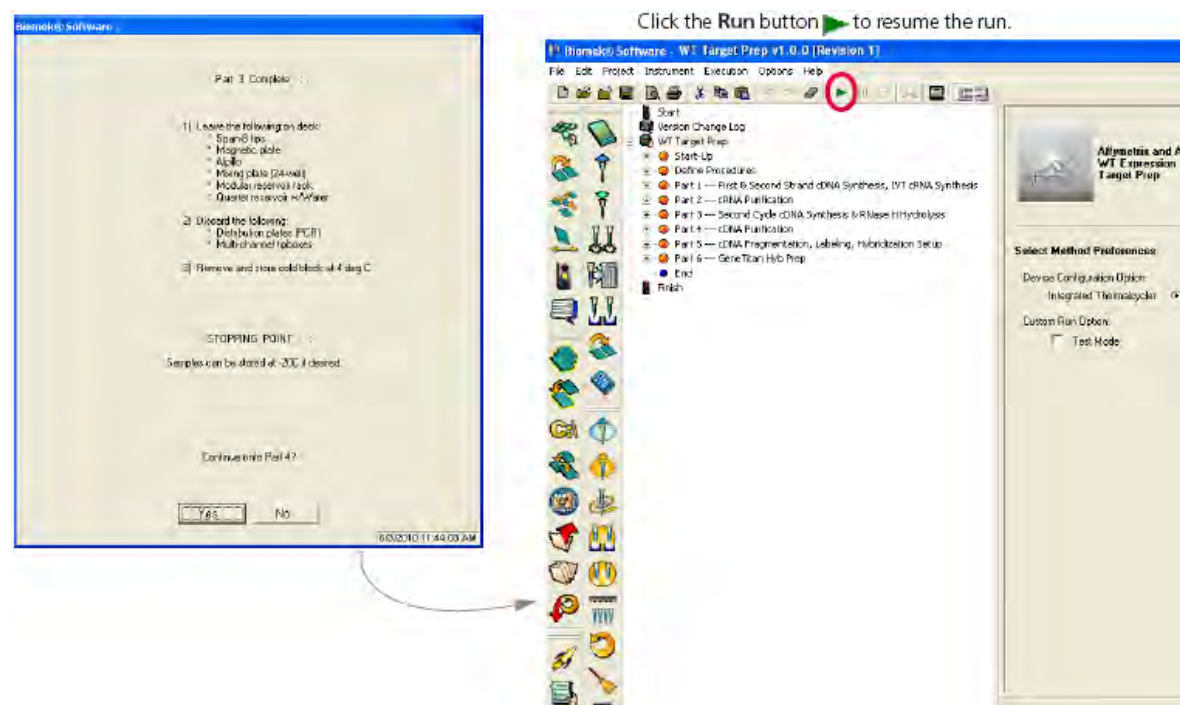


Figure 42 Stopping/resuming the run.

Part 4—cDNA Purification

After the cDNA synthesis is complete, the cDNA is purified to remove enzymes, salts, and unincorporated nucleotides. At completion, Part 4 yields a Bio-Rad™ Hard-Shell™ 96-Well PCR Plate with quantified and normalized cDNA.

Approximate time required for Part 4

Part 4—cDNA Purification	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	15	15	15	15
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: cDNA purification	65	65	65	82
Normalization	4	4	4	11
Part 4 total time	84 1.40 hours	84 1.40 hours	84 1.40 hours	108 1.80 hours

Reagents and labware required for Part 4

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 32 Part 4 reagent requirements.

Per run	Description
1	Invitrogen™ WT Expression Kit, high throughput 4440537 , 24 reactions ^[1] or 4440536 , 96 reactions Components needed: • Nucleic Acid Binding Buffer Concentrate • Nucleic Acid Binding Beads • Nucleic Acid Wash Solution Concentrate • Elution Solution • Nuclease-free Water • Ethanol, 200 proof
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

^[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Note: All components and reagents, except beads, for Part 2 and Part 4 should be at room temperature. Allow samples to sit at room temperature for at least 10 minutes. The Bead Binding Buffer will precipitate if stored at 4°C. Ensure that the binding buffer and beads are at room temperature prior to starting the procedure.

Table 33 Part 4 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ Span P50 Pipette Tips (magenta) Beckman Coulter™, A21586
2 boxes	2 boxes	2 boxes	2 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
4	4	4	4	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™ HSP9631 (blue) or HSP9601 (white), or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
2	2	2	2	Costar™ U-bottom 96-well, Polystyrene Plate Costar™, 3798, or part of 901646
1	1	1	1	ABgene™ 96 Square Well Storage Plate ABgene™, AB-0932, or part of 901646
1	1	1	1	Greiner Bio-One™ UV-Star™ Plate Greiner Bio-One™, 655801, or part of 901646
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790

Table 33 Part 4 labware requirements. *(continued)*

8 reactions	16 reactions	24 reactions	96 reactions	Description
2	2	2	2	Quarter Reservoir Divided by Width Beckman Coulter™, 372792
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Perform a prerun check of the Biomek™ FXP TPE System

Note: The prerun check is not required if you continue on to Part 4 without stopping the method at the end of Part 3.

The following actions are the same as described under “Prepare the Biomek™ FXP TPE System” on page 43. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek™ FXP Target Prep Express Automated Workstation.

1. Power on the workstation and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek™ Software.
4. If applicable, close the DNA Engine™ PTC-200 Thermal Cycler lid.
5. Home all axes.

Prepare the samples and reagents

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

Thaw and prepare the reagents

Thaw the following reagents from the WT Expression Kit on the bench top at room temperature.

- Binding Beads
- Binding Beads Buffer
- Wash Solution

IMPORTANT! Add ethanol to wash solution prior to use. Follow the instructions on the bottle.

- Elution Buffer
- Vortex and centrifuge the reagents, then place on ice.

Continue the run—Part 4—cDNA Purification

If you are beginning the run at Part 4, click **Run**  below the menu bar, then make selections from the **Run Settings Options** window. Then begin Part 4 at step 2.

1. If prompted, place a new box of tips as indicated in the message prompt. When ready to continue click **OK**.
2. The **Labware & Reagent Setup** window appears (Figure 43).

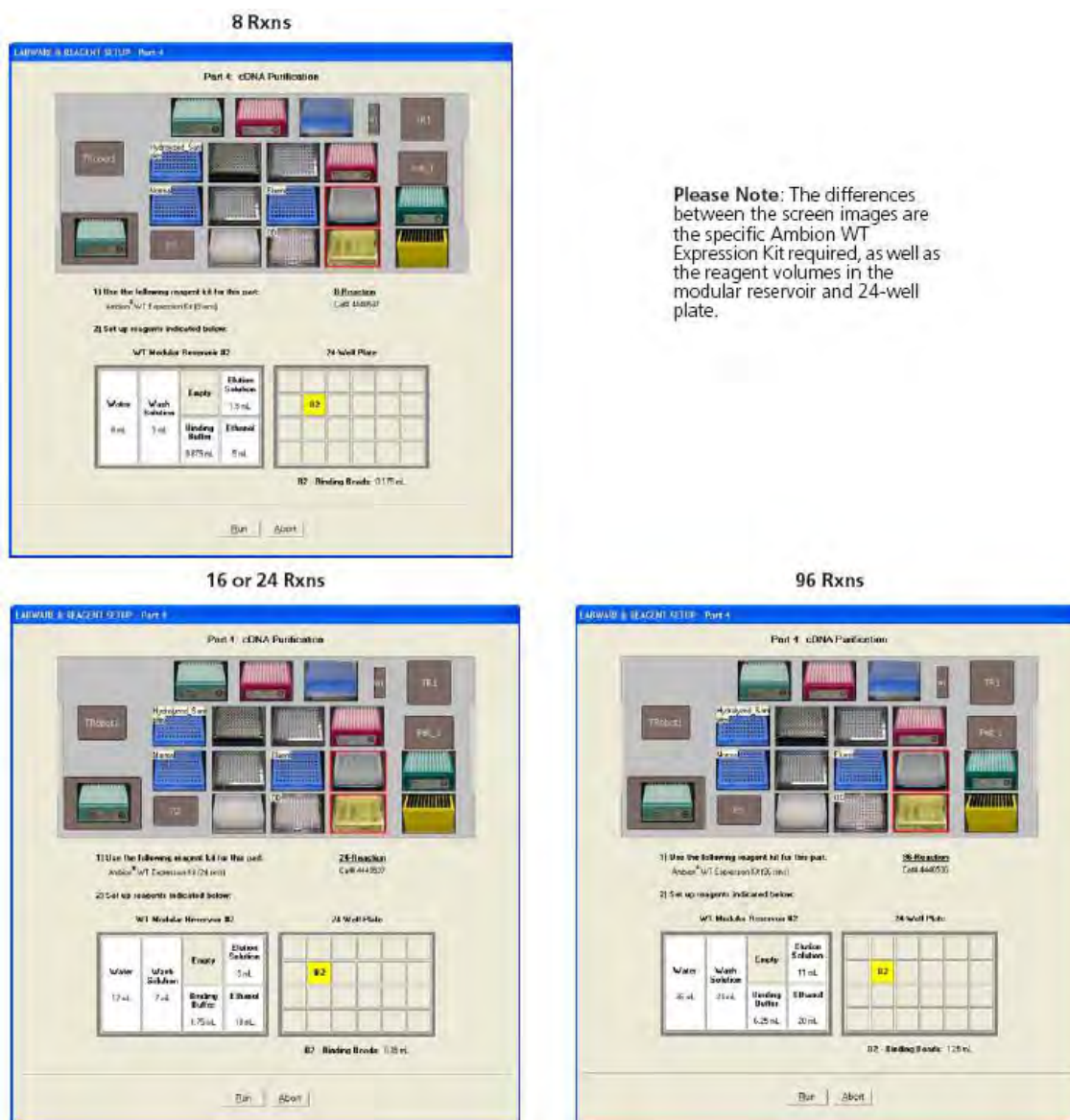


Figure 43 Part 4 labware and reagent setup.

IMPORTANT! At position P11 there is an Alpipto™ Plate Cushion under the 24-well mixing plate. See Table 34 for labware setup details.

3. Set up the 24-well plate and Modular Reservoir, and place labware on the deck as directed in the following figures and table:

- Figure 43— Illustration of the deck layout
- Figure 44— Pipette the correct reagent volumes in the 24-well plate

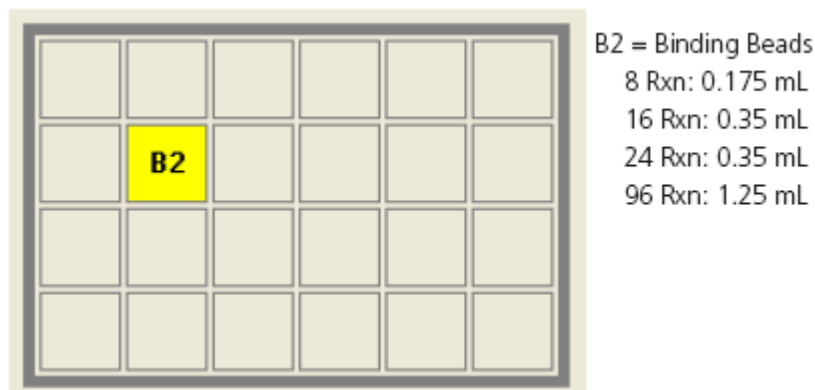


Figure 44 24-well plate for Part 4 deck setup.

- Figure 45— location names of empty deck positions

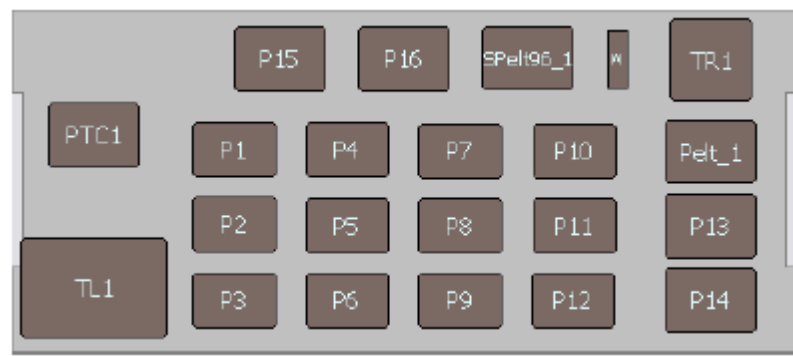


Figure 45 Empty deck positions.

- Table 34 — Pipette the correct reagent volumes in the modular reservoir. See Table 34 detailing the labware and modular reservoir placement for the deck layout.

Table 34 Labware and reagent locations on the deck for Part 4.

Position on deck ^[1]	Labware	Use	Reagent or samples
TL1	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Beads	
P1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Hydrolyzed Sample	Hydrolyzed Sample from Step 3
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Normal	
P4	Alpaqua™ Magnetic Plate	Bead Magnet	

Table 34 Labware and reagent locations on the deck for Part 4. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples			
P5	Costar™ Polystyrene 96-well, U-bottom Plate	Bead Binding				
P6	ABgene™ 96 Square Well Storage Plate	EtOH-Waste				
P7	Costar™ Polystyrene 96-well, U-bottom Plate	Wash Solution				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Eluent				
P9	Greiner Bio-One™ UV-Star™ Plate	OD				
P10	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—Span8				
P11	Alpillo™ Plate Cushion with 24-well mixing plate	Mixing	See Figure 44 for setup			
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4
			Water	Wash Solution	Empty	Elution Solution

Table 34 Labware and reagent locations on the deck for Part 4. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples			
P12	– Quarter module reservoir in position 1 and 2 – Quarter module reservoir divided by width in positions 3 and 4 – Pipette water into reservoir 1 – 8 reactions = 6 mL – 16 reactions = 12 mL – 24 reactions = 12 mL – 96 reactions = 36 mL – Pipette wash solution into reservoir 2 – 8 reactions = 3 mL – 16 reactions = 7 mL – 24 reactions = 7 mL – 96 reactions = 24 mL – Pipette binding buffer into the bottom half of reservoir 3 – 8 reactions = 0.875 mL – 16 reactions = 1.75 mL – 24 reactions = 1.75 mL – 96 reactions = 6.25 mL – Pipette elution solution into the top half of reservoir 4 – 8 reactions = 1.5 mL – 16 reactions = 3 mL – 24 reactions = 3 mL – 96 reactions = 11 mL – Pipette Ethanol into the bottom half of reservoir 4 – 8 reactions = 5 mL – 16 reactions = 10 mL – 24 reactions = 10 mL – 96 reactions = 20 mL	Reagent Reservoirs	Water	Wash Solution	Binding Buffer	Isopropanol
P13	Biomek™ Span P250 Barrier Tips ^[2] (green)	P200—Span8				
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000—Span8				
P15	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Elution				

Table 34 Labware and reagent locations on the deck for Part 4. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples
p16	Biomek™ AP96—P50 Barrier Tips (magenta)	P50—OD	
Spelt96_1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Elution Buffer	

^[1] See Figure 45 for empty deck positions.

^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

- When ready, click **Run** in the **Labware & Reagent Setup** window (Figure 43).
A message to purge the Pod2 syringe lines appears (Figure 46).

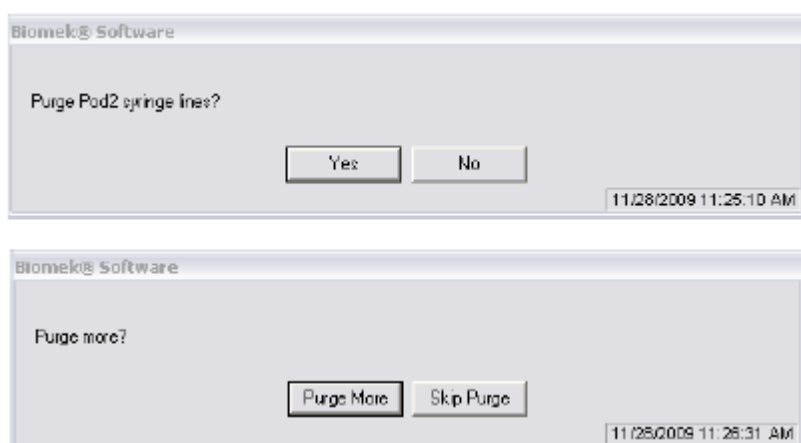


Figure 46 Prompts to purge Pod2 syringe lines.

- If bubbles are present in the Pod2 syringe lines, click **Yes** to begin purging.
If necessary, click **Purge More** in the next prompt (Figure 46). If no further purging is required, click **Skip Purge**. The run proceeds.
At this point the Biomek™ FX^P TPE System begins the cDNA Purification method without user intervention. After a blank OD plate is prepared (contains diluent only), the following message appears prompting you to read the plate.



6. Remove the OD plate from position P9 on the deck.
7. Read background OD in a plate reader at 260 nm.
8. Export or save background OD data for later use.
9. After the plate is read, remove it from the plate reader and place it on deck position P9. Click **OK** in the prompt (Figure 47) to proceed with sample transfer into the OD plate.



Figure 47 Prompt to read OD plate background.

10. Click **OK** after removing OD plate from P9 when prompted again to read OD plate

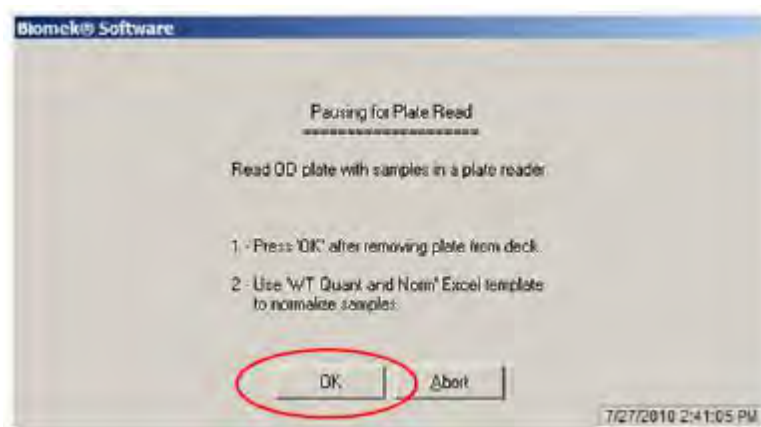


Figure 48 .

Note: The WT Quant and Norm.xls template (Figure 49) opens in the background waiting for OD data.

11. Read sample OD in plate reader at 260 nm.
12. Export or save sample OD data.

13. Return to the WT Quant and Norm.xls template (Figure 49).

Quantitation and Normalization Calculations Template

1. Import or Paste Quantitation Plate Data

Import Form Data

Quant Parameters

Quant Values	
Absorbance 260	TRUE
Sample Volume	3
Blank Volume	197
Biomek Variable	
Normalization Vol	24
Normalization Conc	0.555416667
Sample Type	cDNA
Normal UV Plate	TRUE
Sample Vol	35
Number of Samples	24

2. Check Normalization Values

Sample Plate Information	
Highest Well Conc	0.00 ug/mL
Lowest Well Conc	0.00 ug/mL
Average of All Wells	0.00 ug/mL
Sample Volume Left	30.00 uL

Volume: 24 uL
Concentration: 0.55542 ug/uL

3. Export Normalization CSV file:

Export Data to Biomek

Figure 49 WT Quant and Norm.xls template.

14. Click **Import Form Data** in the Quantitation and Normalization Calculations Template. The Raw Data Form worksheet is shown (Figure 50).

RAW DATA FORM

1. Paste Raw Background Data

2. Paste Raw Sample Data

Adjusted Sample Data

Figure 50 Raw data form worksheet.

15. Copy and paste background OD data saved in step 8 into Section 1 of the Raw Data Form (Figure 51).

RAW DATA FORM

1. Paste Raw Background Data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
B	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
C	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
D	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
E	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
F	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
G	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
H	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635

2. Paste Raw Sample Data

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Adjusted Sample Data

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Section 1: Paste Raw Background Data here

Figure 51 Paste raw background data.

16. Copy and paste sample OD data saved in step 12 into Section 2 of the Raw Data Form (Figure 52).

RAW DATA FORM

1. Paste Raw Background Data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
B	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
C	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
D	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
E	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
F	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
G	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635
H	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635	0.0635

2. Paste Raw Sample Data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
B	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
C	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
D	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
E	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
F	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
G	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104
H	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104	0.1104

Adjusted Sample Data

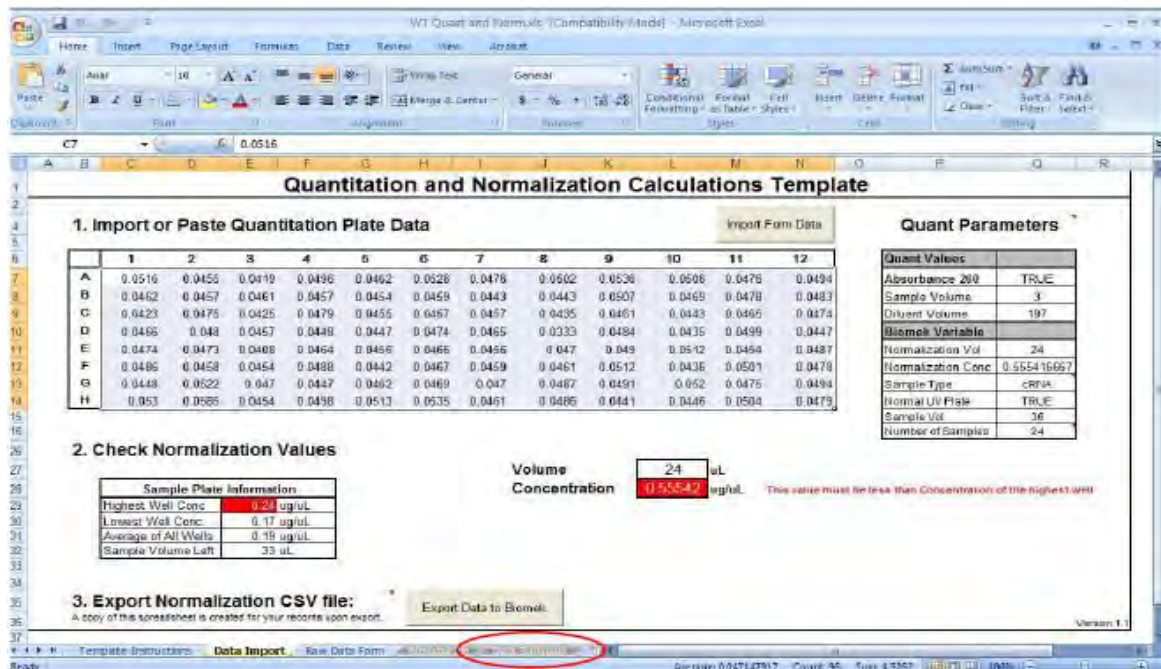
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
B	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
C	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
D	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
E	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
F	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
G	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478
H	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478	0.0478

Section 2: Paste Raw Sample Data here

Automatically Calculated

Figure 52 Paste raw sample data.

17. Click **Copy Adjusted Data to Template** to copy the adjusted OD values to the template.



18. Check the yield by clicking the **Data Summary** tab. The next step requires a minimum 5.5 µg in 31.2 µL volume.



CAUTION! Normalizing below the required minimum sample concentration at this point is not recommended and may yield undesired results. The normalization process can proceed, if desired.

- Samples that are outside the range of normalization (20 µg to 200 µg) will be carried through the remainder of the procedure but will not be properly normalized. Hybridization results from improperly normalized samples may not be valid.
- Low OD readings may be due to bubbles in sample wells. Check the plate and inspect the UV plate for bubbles. If bubbles are present, carefully remove them using a clean pipette tip.

19. In the **Data Import** tab, click **Export Data to Biomek** in the Quantitation and Normalization Calculations Template. If any of the samples do not meet the minimum concentration a warning message appears (Figure 53). Click **Yes** in the prompt that appears (Figure 54).

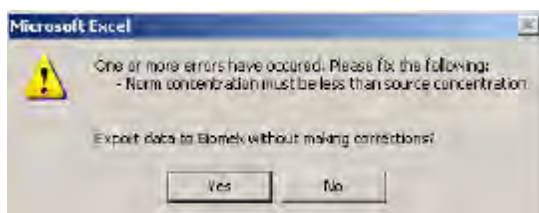


Figure 53 Warning message.

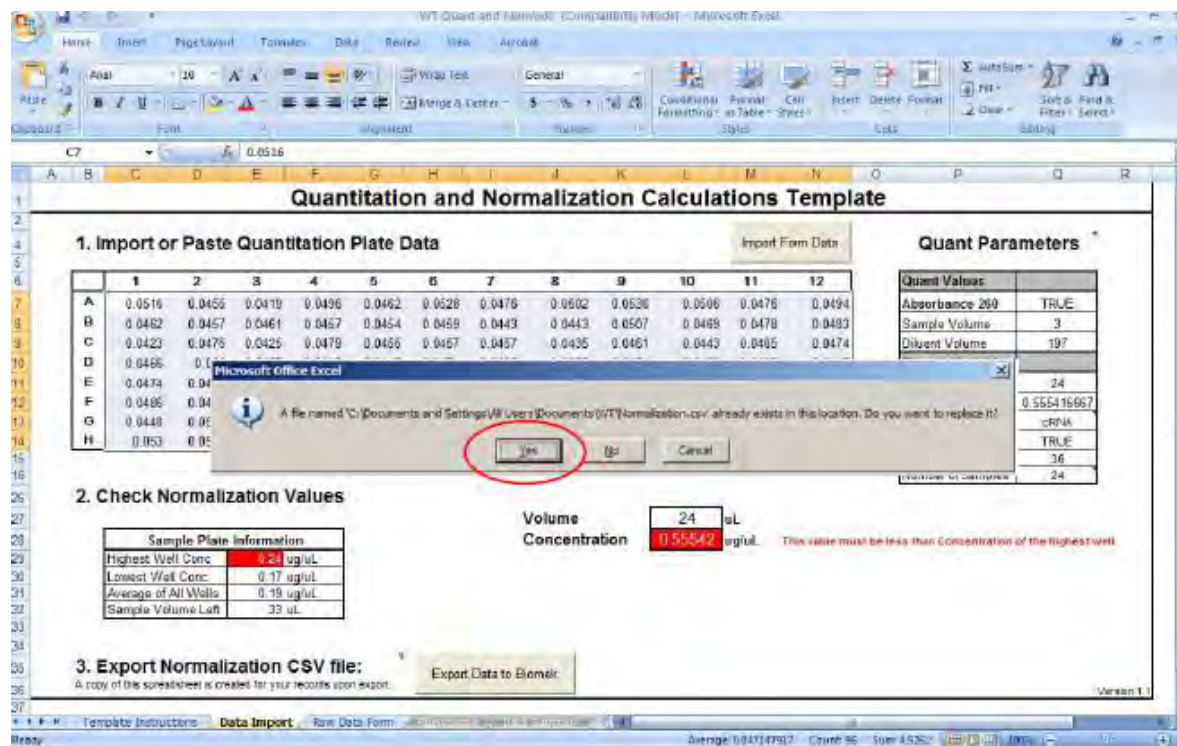


Figure 54 Quantitation and normalization calculations template.

The next dialog box that appears (Figure 55) enables you to save a copy of the background-corrected data and the parameters used to determine sample normalization. Saving a backup copy is optional.

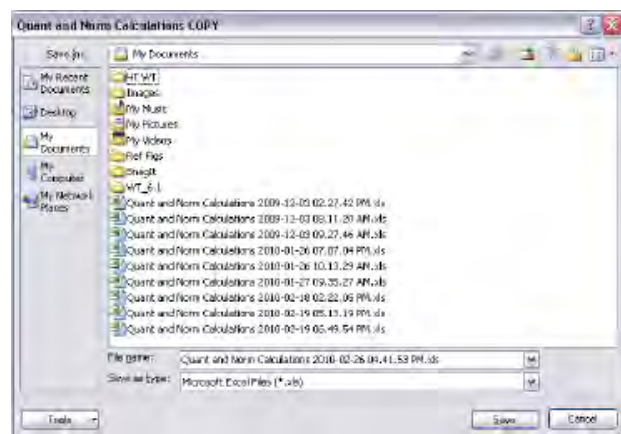


Figure 55 Prompt to save a copy of the OD data and normalization parameters.

20. To save a backup copy of the data and normalization parameters:
 - a. Confirm the default folder or select a new folder.
 - b. Confirm the default file name or enter a new file name.

21. Click **Save**.

The sample volumes and concentrations are normalized using elution buffer as the diluent. The Quantitation and Normalizations Template closes.

22. Remove the OD plate from plate reader and discard.

When Part 4 is complete, the message shown in Figure 56 appears.

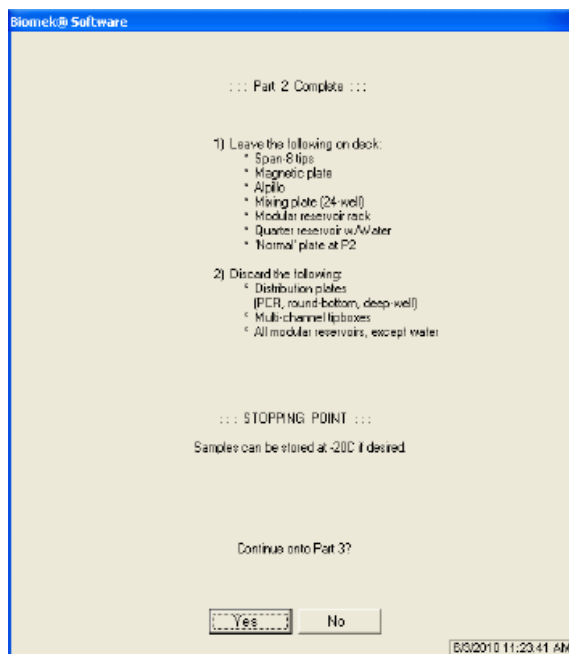


Figure 56 Message at completion of Part 4.

STOPPING POINT After Part 4 is complete, the samples can be stored at -20°C .

23. Follow the instructions in the message.

- a. Discard used labware and reagents.
- b. Discard used multichannel tips.
- c. Leave 'Normal' plate at P2
- d. Leave Span8 tips in current locations.

24. To continue on to Part 5, click **Yes**.

25. If you do not want to continue to run, click **No**. When ready to resume ensure that clean, fresh Span-8 tip boxes are placed on the deck and click **Run**  in the window that appears (Figure 57).

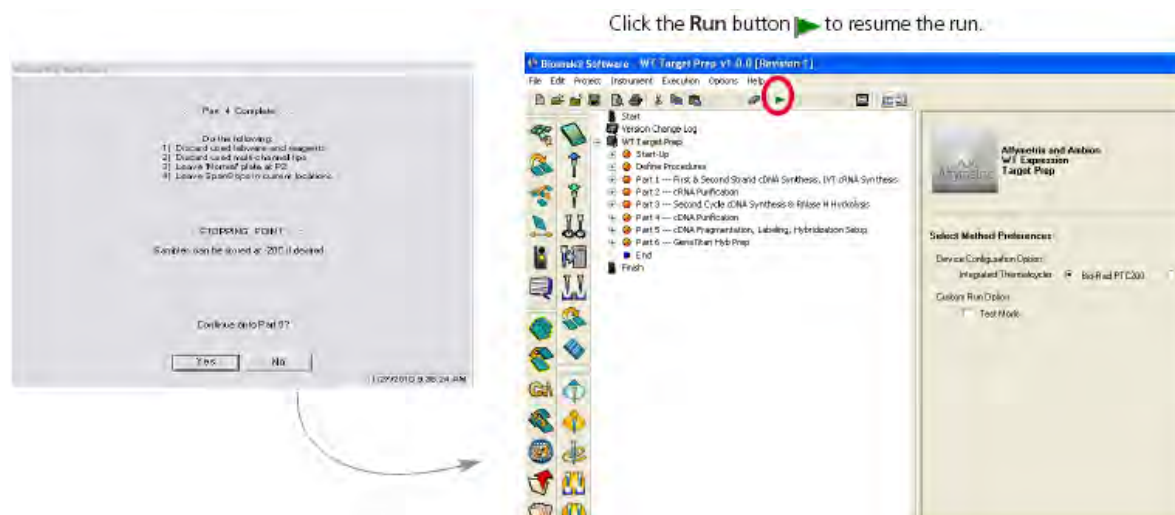


Figure 57 Stopping/Resuming the run.

Part 5—cDNA Fragmentation, Labeling, and Hybridization Setup

Purified, sense-strand cDNA is fragmented by UDG and APE 1, then labeled by TdT. Fragmented and labeled ss-cDNA is then prepared for hybridization. At completion, Part 5 yields hybridization-ready target in either a FrameStar™ Break-A-Way PCR Plate (for cartridges) or a Bio-Rad™ Hard-Shell™ 96-Well PCR Plate (for plates).

Approximate time required for Part 5

Part 5—cDNA Fragmentation, Labeling, and Hybridization Setup	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	10	10	10	10
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: cDNA fragmentation	4	5	5	13
Thermal cycler: fragmentation method	64	64	64	64
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: labeling	4	5	5	13
Thermal cycler: labeling method	72	72	72	72

(continued)

Part 5—cDNA Fragmentation, Labeling, and Hybridization Setup	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps: hybridization setup	10	11	11	13
GeneChip™ cartridge array hybridization setup	N/A	12	12	14
GeneChip™ array plate hybridization setup				
Part 5 total time for cartridge array	164 2.73 hours	167 2.78 hours	167 2.78 hours	185 3.08 hours
Part 5 total time for array plate	N/A	168 2.80 hours	168 2.80 hours	186 3.10 hours

Reagents and labware required for Part 5

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 35 Part 5 reagent requirements.

Per run	Description
1	GeneChip™ HT WT Terminal Labeling Kit 901555 (24 reactions ^[1]), 901518 (96 reactions) Components needed: • 10X cDNA Fragmentation Buffer • UDG, 10 U/uL • APE 1, 1000 U/uL • 5X TdT Buffer • TdT, 30 U/uL • DNA Labeling Reagent, 5 mM • RNase-free Water
see Table 36	GeneChip™ Hybridization Control Kit 900454 (30 reactions) or 900457 (150 reactions)
see Table 36	GeneChip™ Hybridization, Wash, and Stain Kit, 900720 (30 reactions) or GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates, 901622 (96 reactions)
	Nuclease-free Water

Table 35 Part 5 reagent requirements. (continued)

Per run	Description
	DNAZap™ PCR DNA Degradation Solutions
	RNaseZap™ RNase Decontamination Wipes

[1] One 24-reaction kit is good for (2) 8-reaction runs, (1) 16-reaction run or (1) 24-reaction run.

Table 36 Part 5 kit requirements.

Sample format				
	8 ^[1] , 16, and 24 reactions		96 reactions	
Array option:	Cartridge	Plates	Cartridge	Plates
Hybridization, wash, and stain kit	30 reactions 900720	96 reactions 901622	4 x 30 reactions 900720	96 reactions 901622
Hybridization control kit	30 reactions 900454	30 reactions 900454	150 reactions 900457	150 reactions 900457

[1] Cartridge option only for 8-sample reaction.

Table 37 Part 5 labware requirements for cartridges.

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
1	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
1 box	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
1 box	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.
3 boxes	3 boxes	3 boxes	3 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
3	3	3	3	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646

Table 37 Part 5 labware requirements for cartridges. (continued)

8 reactions	16 reactions	24 reactions	96 reactions	Description
1	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
1	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
1	1	1	1	Greiner Bio-One™ U-Bottom 96 well Polypropylene Plate Greiner Bio-One™, 650201, or part of 901646
1	1	1	1	24-Position Tube Rack Beckman Coulter™, 373661 (24-position rack), 373696 (white 10-mm insert)
1	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
1	1	1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
2	2	2	2	Quarter Reservoir Beckman Coulter™, 372790
1	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Table 38 Part 5 labware requirements for plates.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	1	1	1	Reagent Cold Block, kept in 4°C storage Beckman Coulter™, A83054
N/A	1	1	1	Reagent Cold Block template, kept in 4°C storage Part of 90-0931
N/A	1 box	1 box	1 box	Biomek™ Span P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
N/A	1 box	1 box	1 box	Biomek™ Span P250 Barrier Tips (green) Beckman Coulter™, 379503 The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

Table 38 Part 5 labware requirements for plates. *(continued)*

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	3 boxes	3 boxes	3 boxes	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
N/A	6	6	6	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
N/A	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™, MSL-2032, or part of 901646
N/A	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931
N/A	1	1	1	24-Position Tube Rack Beckman Coulter™, 373661 (24-position rack), 373696 (white 10-mm insert)
N/A	1	1	1	Alpillo™ Plate Cushion Part of 90-0931
N/A		1	1	Agilent™ 24-well mixing plate Thomas Scientific™, EK-2053, or part of 901646
N/A	1	1	1	Quarter Reservoir Beckman Coulter™, 372790
N/A	1	1	1	Reservoir Frame Beckman Coulter™, 372795

Perform a prerun check of the Biomek™ FX^P TPE System

Note: The prerun check is not required if you continue on to Part 5 without stopping the method at the end of Part 4.

The following actions are the same as described under “Prepare the Biomek™ FX^P TPE System” on page 43. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek™ FX^P Target Prep Express Automated Workstation.

1. Power on the workstation and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek™ Software.
4. If applicable, close the DNA Engine™ PTC-200 Thermal Cycler lid.
5. Home all axes.

Prepare the samples and reagents

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

Thaw and prepare the reagents

To thaw and prepare the reagents:

1. Thaw the following reagents from the GeneChip™ HT WT Terminal Labeling Kit on the bench top at room temperature.
 - Fragmentation Buffer
 - DNA Labeling Reagent
 - TdT Buffer
2. Thaw the following reagents from the GeneChip™ Hybridization Control Kit on the bench top at room temperature.
 - 3 nM Control Oligo™ B2
 - 20X Hybridization Controls

3. (Cartridge arrays) Remove the following reagents from the GeneChip™ Hybridization, Wash, and Stain Kit.
 - 2X Hybridization Mix
 - Nuclease-free Water
 - DMSO (Thaw on the bench top at room temperature)
4. (Array plates) Remove the following components from the GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates.
 - 5X WT Hyb Add 1
 - 15X WT Hyb Add 4
 - 2.5X WT Hyb Add 6
5. Vortex and centrifuge the reagents, then place on ice.

Continue the run—Part 5—cDNA Fragmentation, Labeling, and Hybridization Setup

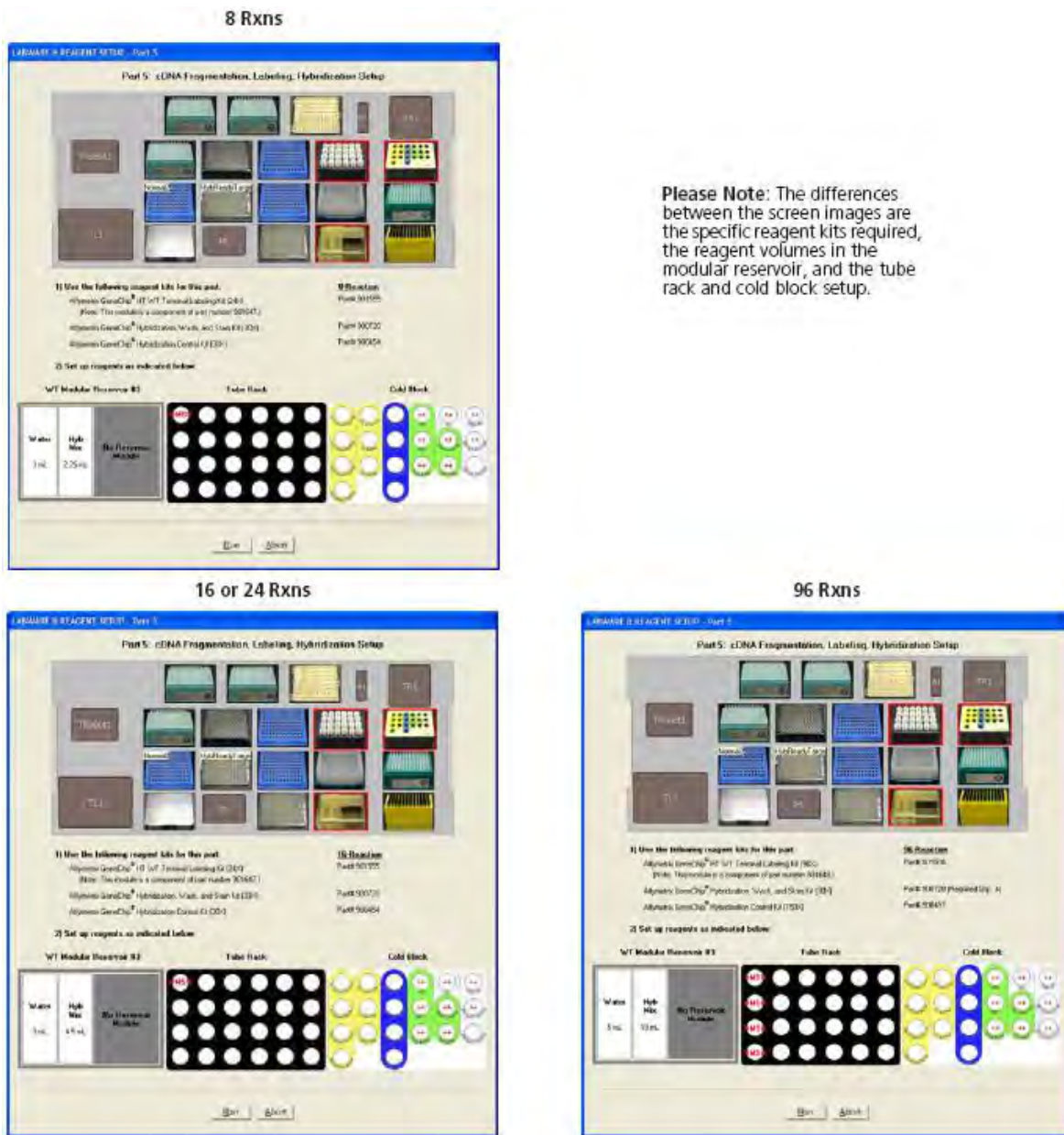
Part 5: cDNA fragmentation, labeling, and hybridization setup

If you are beginning the run at Part 5, click **Run**  below the menu bar, then make selections from the **Run Settings Options** window. Begin Part 5 at step 2.

1. If prompted, place a new box of tips as indicated in the message prompt. When ready to continue click **OK**.
2. If running cartridge arrays, go to “Part 5 deck and reagent setup for cartridge arrays” on page 104. If running array plates, go to “Part 5 deck and reagent setup for array plates” on page 108.

Part 5 deck and reagent setup for cartridge arrays

1. The **Labware & Reagent Setup** window for your run appears (Figure 58).



Please Note: The differences between the screen images are the specific reagent kits required, the reagent volumes in the modular reservoir, and the tube rack and cold block setup.

Figure 58 Part 5 labware and reagent setup for cartridge arrays.

IMPORTANT! At position P11 there is an Alpipto™ Plate Cushion under the 24-well mixing plate. See Table 39 for labware setup details.

2. Set up the modular reservoir, and place labware on the deck as directed in the following figures and table:

- Figure 58—Illustration of the deck layout for cartridge arrays
- Figure 59—Location names of empty deck positions

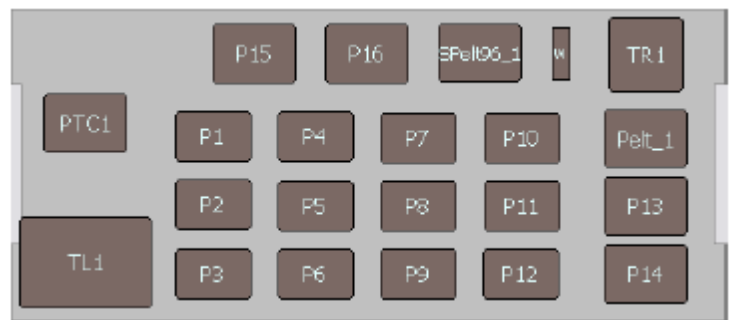


Figure 59 Empty deck positions.

- Table 39—Pipette the correct reagent volumes in the modular reservoir. See Table 39 detailing the labware and modular reservoir placement for the deck layout.

Table 39 Labware and reagent locations on the deck for Part 5: cDNA fragmentation, labeling, and hybridization setup—cartridge.

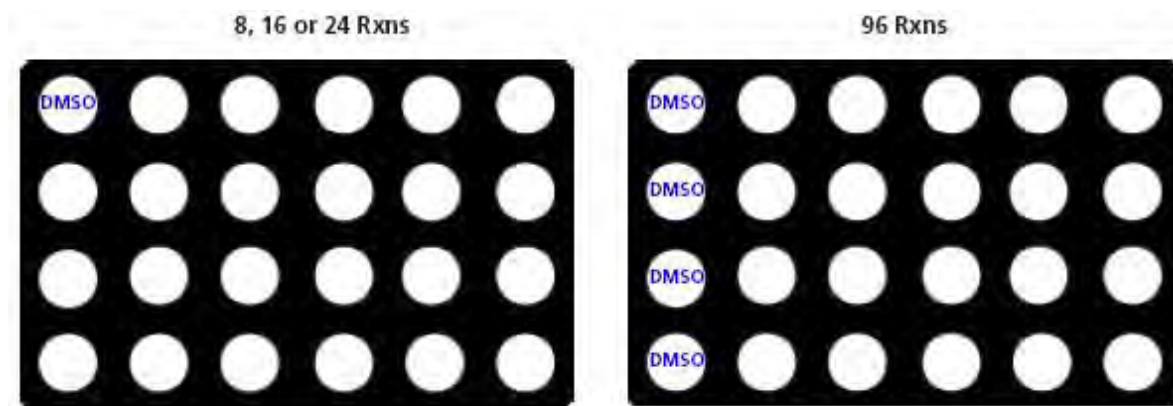
Position on deck ^[1]	Labware	Use	Reagent or samples			
P1	Biomek™ AP96 – P250 Barrier Tips (aqua)	P200-Frag				
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Normal	Normalized cDNA sample from Part 4			
P3	Auto-sealing Microplate Lid with Microseal™ P Pad	Lid				
P4	Alpaqua™ Magnetic Plate	Magnetic Plate				
P5	Greiner Bio-One™ U-Bottom, Polypropylene Plate	Hybridization-ready target				
P7	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Frag Dist.				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Label Dist.				
P9	Greiner Bio-One™ U-Bottom, Polypropylene Plate	Hybridization Cocktail Dist.				
P10	24-Position Tube Rack	Hybridization Reagents	Setup instructions are provided in Step 3			
P11	Alpillo™ Plate Cushion with 24 well mixing plate	Mixing				
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4

Table 39 Labware and reagent locations on the deck for Part 5: cDNA fragmentation, labeling, and hybridization setup—cartridge. (continued)

Position on deck ^[1]	Labware	Use	Reagent or samples		
P12	- Quarter module reservoir in position 1 and 2 - Positions 3 and 4 are empty - Pipette water into reservoir 1 8 reactions = 3 mL 16 reactions = 3 mL 24 reactions = 3 mL 8 reactions = 3 mL 96 reactions = 5 mL - Pipette hybridization buffer into reservoir 2 8 reactions = 3 mL 16 reactions = 7 mL 8 reactions = 3 mL 24 reactions = 7 mL 96 reactions = 24 mL	Reagent Reservoirs	Water	2X Hyb Mix	Empty
Pelt_1	Reagent Cold Block	Cold Block	Setup instructions are provided in Step 4		
P13	Biomek™ Span P250 Barrier Tips (green) ^[2]	P200-Span8			
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000-Span8			
P15	Biomek™ AP96 – P250 Barrier Tips (aqua)	P200-Label			
P16	Biomek™ AP96 – P250 Barrier Tips (aqua)	P200-Hyb			
Spelt96_1	Peltier Adapter (installed on deck)				

^[1] See Figure 59 for empty deck positions.^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

3. Set up the 24-position tube rack as shown in Figure 60, depending on your sample format.

**Figure 60** Tube rack setup for cartridge arrays.

4. Set up the reagent cold block as shown in Figure 61 A or B, depending on your sample format.

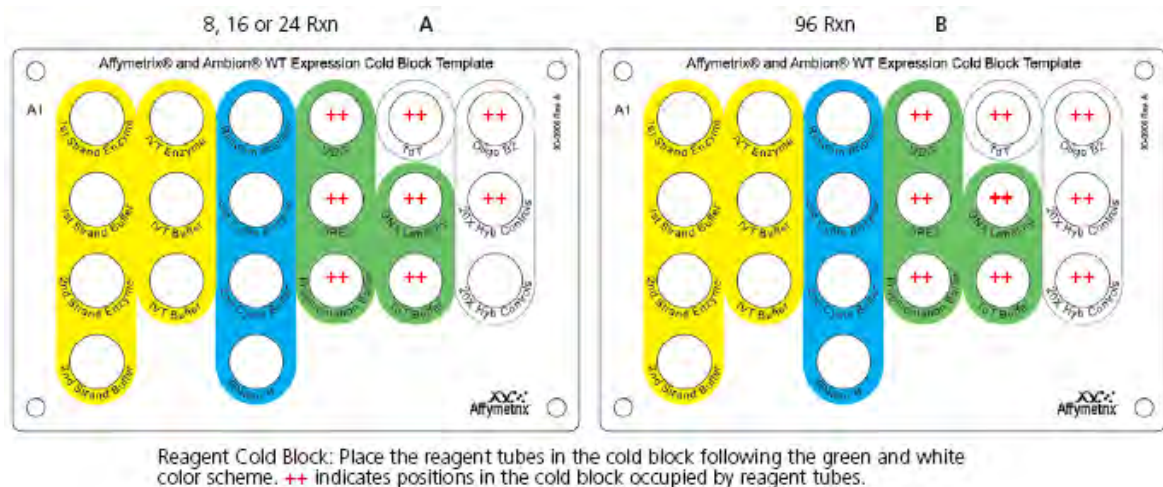


Figure 61 Reagent cold block setup for 8, 16, 24, and 96 reactions for Part 5 cartridge arrays.

- a. Place the reagent cold block template onto the cooled (4°C) cold block.
- b. Uncap the Fragmentation and DNA Labeling Reagent tubes and insert them into the cooled reagent cold block following the green and white color code of the template (Figure 61).
- c. Uncap the thawed 3 nM Control Oligo™ B2 and 20X Hybridization Controls tubes and insert them into the cooled reagent cold block following the white color code of the template (Figure 61).
- d. Confirm that the Static Peltier is set to 4°C, then place the cold block on deck position Pelt_1.

IMPORTANT! Before loading the tubes, mix buffer solutions by vortexing. If a precipitant is present in the buffer, dissolve by placing in a heat block at 40°C, vortex, and return to heat block until dissolved. Mix enzyme solutions by gently flicking the reagent tube a few times. Briefly centrifuge ALL tubes before inserting them into the reagent cold block. Inspect for bubbles. Wipe the bottom of the reagent cold block before placing it on the deck.

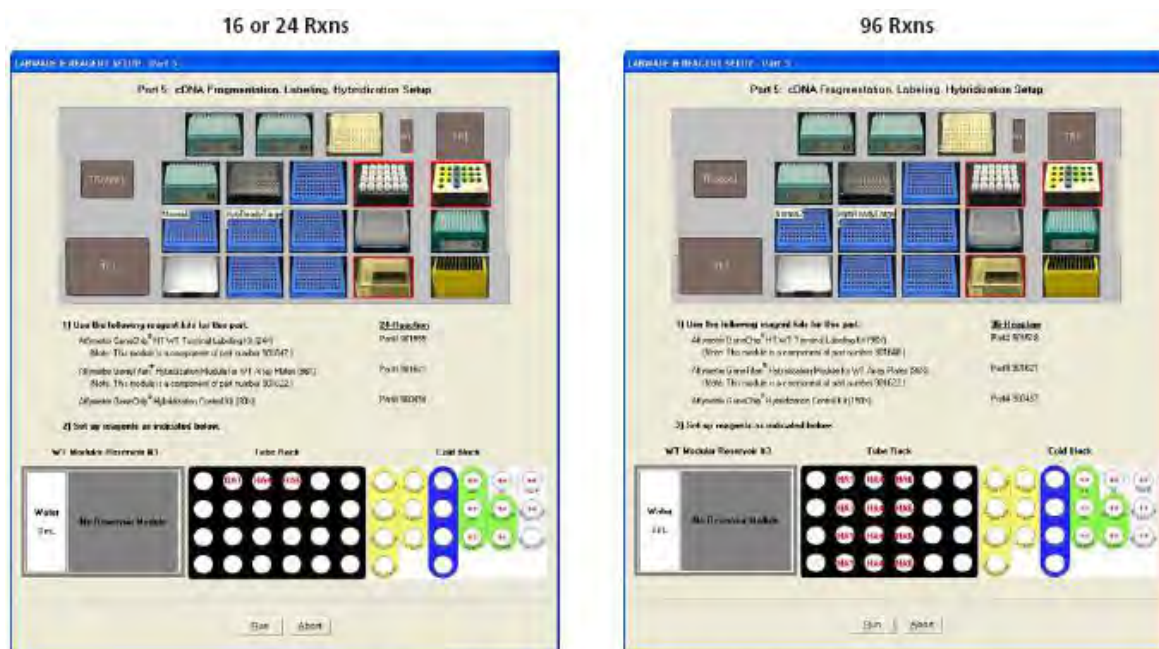


CAUTION! DMSO freezes at 4°C. Ensure that DMSO is completely thawed before placing it in the tube rack.

5. Continue at Step 1 in “Continue the run after deck and reagent setup” on page 111.

Part 5 deck and reagent setup for array plates

1. The **Labware & Reagent Setup** window for your run appears (Figure 62).



Please Note: The differences between the screen images are the specific reagent kits required, the reagent volumes in the modular reservoir, and the tube rack and cold block setup.

Figure 62 Part 5 labware and reagent setup for array plates.

IMPORTANT! At position P11 there is an Alpipto™ Plate Cushion under the 24-well mixing plate. See Table 40 for labware setup details.

2. Set up the modular reservoir, and place labware on the deck as directed in the following figures and table:
 - Figure 62—Illustration of the deck layout for array plates
 - Figure 63—Location names of empty deck positions



Figure 63 Empty deck positions.

- Table 40—Pipette the correct reagent volumes in the modular reservoir. See Table 40 detailing the labware and modular reservoir placement for the deck layout.

Table 40 Labware and reagent locations on the deck for Part 5: cDNA fragmentation, labeling, and hybridization setup—plates.

Position on Deck ^[1]	Labware	Use	Reagent or samples			
P1	Biomek™ AP96 – P250 Barrier Tips (aqua)	P200-Frag				
P2	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Normal	Normalized cDNA sample from Part 4			
P3	Auto-sealing Microplate Lid with Microseal™ P Pad	Lid				
P4	Alpaqua™ Magnetic Plate	Magnetic Plate				
P5	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Hybridization-ready target				
P7	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Frag Dist.				
P8	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Label Dist.				
P9	Greiner Bio-One™ U-Bottom, Polypropylene Plate	Hybridization Cocktail Dist.				
P10	24-Position Tube Rack	Hybridization reagents	Setup instructions are provided in step 3			
P11	Alpillo™ Plate Cushion with 24-well mixing plate	Mixing				
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4
	– Quarter module reservoir in position 1 – Positions 2, 3 and 4 are empty – Pipette Water into reservoir 1 – 16 reactions = 3 mL – 24 reactions = 3 mL – 96 reactions = 3 mL		Water	Empty		
Pelt_1	Reagent Cold Block	Cold Block	Setup instructions are provided in step 4			
P13	Biomek™ Span P250 Barrier Tips (green) ^[2]	P200-Span8				
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000-Span8				
P15	Biomek™ AP96—P250 Barrier Tips (aqua)	P200-Label				
P16	Biomek™ AP96—P250 Barrier Tips (aqua)	P200-Hyb				
Spelt96_1	Peltier Adapter (installed on deck)					

^[1] See Figure 63 for empty deck positions.

^[2] The P250 Barrier Pipette Tips are loaded where the user interface shows P200.

3. Set up the tube rack as shown in Figure 64, depending on your sample format.

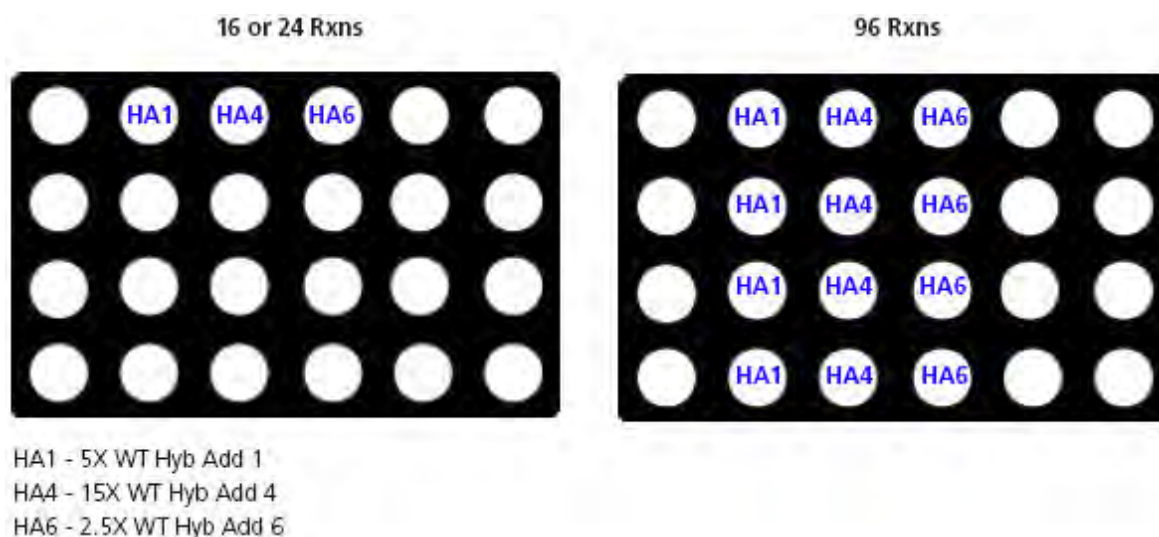


Figure 64 Tube rack setup for array plates.

4. Set up the reagent cold block as shown in Figure 65 A or B, depending on your sample format.

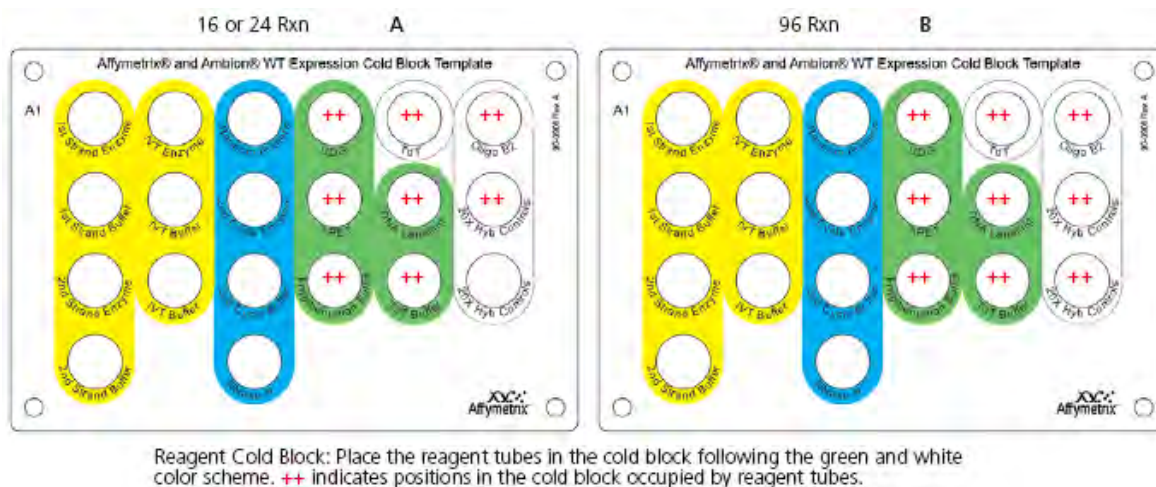


Figure 65 Reagent cold block setup for 16, 24 and 96 reactions for Part 5 array plates.

- Place the reagent cold block template onto the cooled (4°C) cold block.
- Uncap the Fragmentation and DNA Labeling Reagent tubes and insert them into the cooled reagent cold block following the green and white color code of the template (Figure 65).
- Uncap the thawed 3 nM Control Oligo™ B2 and 20X Hybridization Controls tubes and insert them into the cooled reagent cold block following the white color code of the template (Figure 65).

- d. Confirm that the Static Peltier is set to 4°C, then place the cold block on deck position Pelt_1.

IMPORTANT! Before loading the tubes, mix buffer solutions by vortexing. If a precipitant is present in buffer, dissolve by placing in a heat block at 40°C, vortex, and return to heat block until dissolved. Mix enzyme solutions by gently flicking the reagent tube a few times. Briefly centrifuge ALL tubes before inserting them into the reagent cold block. Inspect for bubbles. Wipe the bottom of the reagent cold block before placing it on the deck.

5. Continue at Step 1 in “Continue the run after deck and reagent setup” on page 111.

Continue the run after deck and reagent setup

1. When the deck is ready, click **OK** in the **Labware & Reagent Setup** window (Figure 58 for cartridge arrays or Figure 62 for array plates).

A message to purge the Pod2 syringe lines appears (Figure 66).

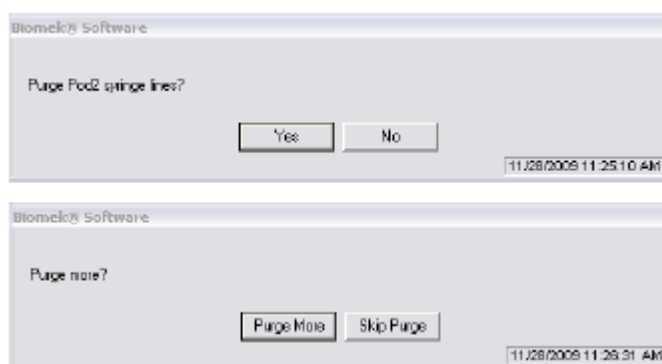


Figure 66 Prompts to purge Pod2 syringe lines.

2. If bubbles are present in the Pod2 syringe lines, click **Yes** to begin purging. If necessary, click **Purge More** in the next prompt (Figure 46). If no further purging is required, click **Skip Purge**. The run proceeds without user intervention. When labeling is complete, the message shown in Figure 41 appears.

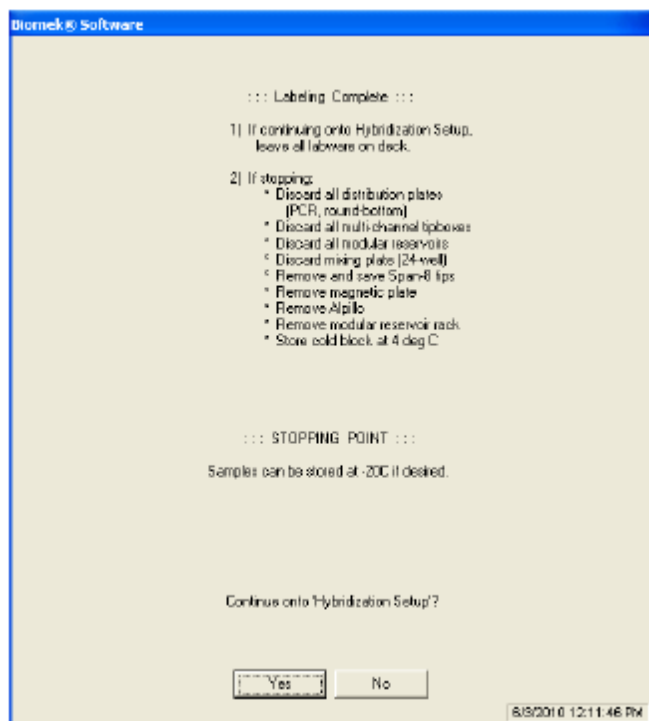


Figure 67 Message at completion of labeling.

3. To continue on to Hybridization Setup, leave all labware on deck and click **Yes**.
4. If you are stopping the run, follow the instructions in the message and click **No**. Store hybridization ready target plate and left over labeled cDNA plate at -20°C for future use.

- When ready to resume, ensure that the thermal cycler lid is closed. Ensure that clean, fresh Span-8 tip boxes are placed on the deck, click **Run**, then click **Run** in the window that appears (Figure 68).

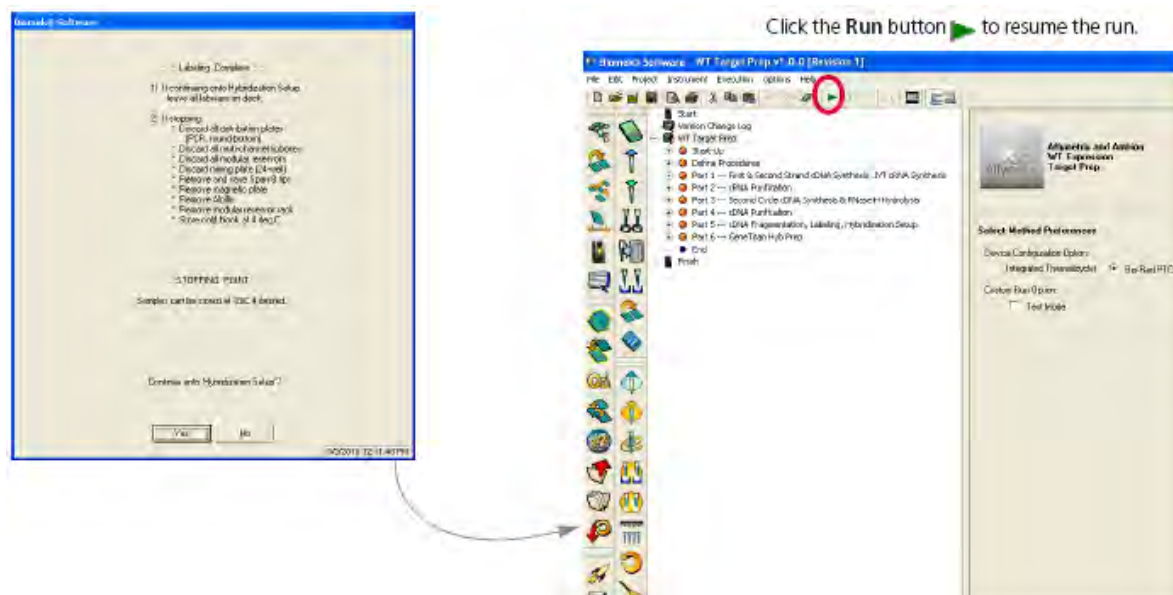


Figure 68 Stopping/resuming the run.
The run proceeds without user intervention.

GeneChip™ cartridge arrays

If you specified the cartridge array sample format, the HT WT Expression Assay is complete when hybridization setup is finished. At the completion of the method, the following message appears.



Figure 69 Message at completion of Part 5 (cartridge array sample format).

For instructions on hybridization, washing and staining, and scanning arrays, see the *GeneChip™ Expression Wash, Stain, and Scan for Expression Cartridge Arrays User Guide*.

GeneChip™ array plates

If you specified the array plate sample format, the method includes Part 6. When hybridization setup is complete, the following message appears.

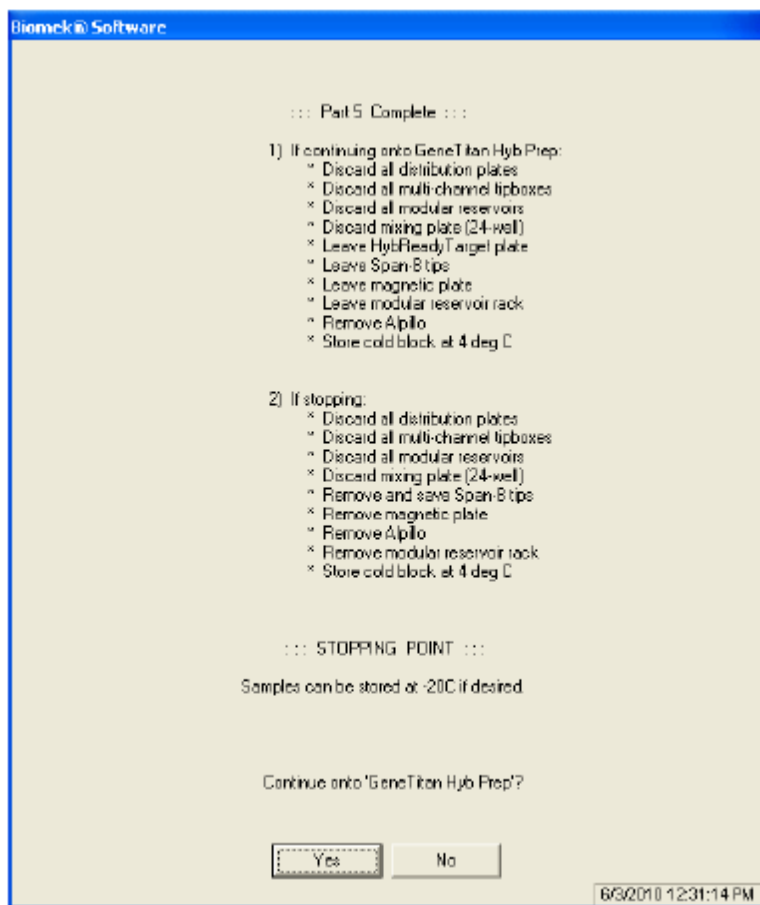


Figure 70 Message at completion of Part 5 (array plate sample format).

1. To continue on to Part 6, follow the instructions in the message, then click **Yes**.
2. If you are stopping the run, follow the instructions in the message, then click **No**. When ready to resume ensure that clean, fresh Span-8 tip boxes are placed on the deck, click **Run**, then click **Run**  in the window that appears (Figure 57).

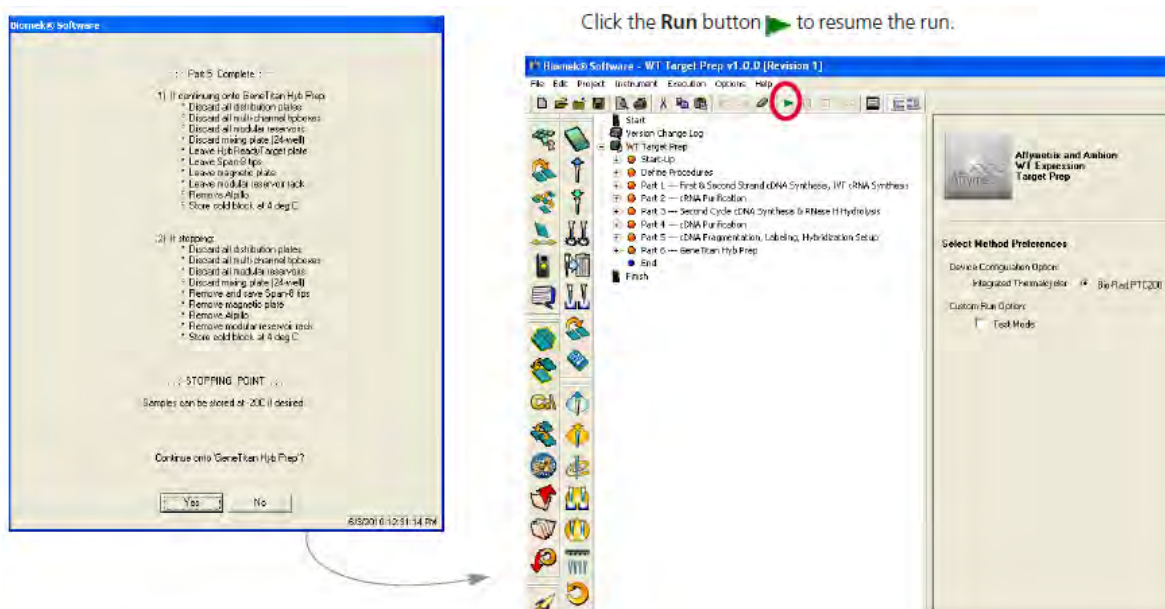


Figure 71 Stopping/resuming the run.

Part 6—GeneTitan™ MC Instrument Hybridization Preparation

At completion, Part 6 yields denatured cDNA in a hybridization plate tray, ready for further processing on the GeneTitan™ MC Instrument. The GeneTitan™ stain trays (Stain 1 to Stain 3) and scan tray are also prepared.

See the *GeneTitan™ Multi-Channel Instrument User Guide* for instructions on the proper handling of plates processed using the GeneTitan™ family of instruments.

Approximate time required for Part 6

Part 6—GeneTitan™ MC Instrument Hybridization Preparation	8 reactions in minutes	16 reactions in minutes	24 reactions in minutes	96 reactions in minutes
Deck setup	N/A	10	10	10
Biomek™ FX ^P Target Prep Express Automated Workstation pipetting steps	N/A	10	30	60
Thermal cycler: denaturation method	N/A	12	12	12
Part 6 total time	N/A	32	52	82 1.37 hours

Reagents and labware required for Part 6

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Table 41 Part 6 reagent requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	√	√	√	Hybridization-ready target plate (from Part 5)
N/A	1	1	1	GeneTitan™ Hybridization, Wash, and Stain Kit for WT Array Plates, 901622 Components needed: • Array Holding Buffer • Stain 1 & 3 • Stain 2

Table 42 Part 6 labware requirements.

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	1 box	1 box	1 box	Biomek™ Span™ P1000 LLS Barrier Tips (yellow) Beckman Coulter™, 987925
N/A	1 box	1 box	1 box	Biomek™ AP96—P250 Barrier Tips (aqua) Beckman Coulter™, 717253
N/A	1	1	1	Auto-sealing Microplate Lid with Microseal™ P Pad Bio-Rad™ MSL-2032, or part of 901646
N/A	1	1	1	Alpaqua™ Magnetic Plate Part of 90-0931

Table 42 Part 6 labware requirements. *(continued)*

8 reactions	16 reactions	24 reactions	96 reactions	Description
N/A	1	1	1	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted Bio-Rad™, HSP9631 (blue) or HSP9601 (white), or part of 901646
N/A	1	1	1	GeneTitan™ Hybridization Tray, 500867 ^[1]
N/A	3	3	3	GeneTitan™ Stain Tray, 501025 ^[1]
N/A	1	1	1	GeneTitan™ Scan Tray, 501006 or 500860 ^[1]
N/A	4	4	4	GeneTitan™ Stain/Scan Tray Lids, 202757 ^[1]
N/A	3	3	3	Quarter Reservoir Beckman Coulter™, 372790
N/A	1	1	1	Reservoir Frame Beckman Coulter™, 372795

^[1] GeneTitan™ trays and lids are provided with the array plate when ordered. The Cat. No. provided here is for identification purposes only.

Perform a prerun check of the Biomek™ FX^P TPE System

Note: The prerun check is not required if you continue on to Part 6 without stopping the method at the end of Part 5.

The following actions are the same as described under “Prepare the Biomek™ FX^P TPE System” on page 43. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek™ FX^P Target Prep Express Automated Workstation.

1. Power on the workstation and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek™ Software.
4. If applicable, close the DNA Engine™ PTC-200 Thermal Cycler lid.
5. Home all axes.

Label GeneTitan™ hybridization and reagent trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ MC Instrument, it is helpful to mark each tray in a way that identifies its contents.

IMPORTANT! It is critical that you write only on the proper locations of the proper sides of hybridization and stain trays. **Do not** write in any other location, because writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan™ MC Instrument, you can also mark the notched corner of the trays and lids.

Thaw and prepare the sample plate

If the sample plate is frozen:

1. Thaw the sample plate.
2. Centrifuge down at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination, remove the seal carefully.

Deionization of GeneTitan™ trays and covers

We recommend the use of the GeneTitan™ ZeroStat AntiStatic Gun (Cat. No. [74-0014](#)) to deionize GeneTitan™ MC Instrument stain trays and covers.

IMPORTANT! Except for the array plate, scan tray, and the hybridization tray, you must deionize all GeneTitan™ stain trays, stain tray covers, and the scan tray cover using an antistatic gun. Always deionize before you fill the trays with reagents and before you place the covers on the trays. Deionization removes the static electricity. Static electricity on the underside of the cover can cause the gripper to lift the tray along with the tray cover and can result in an aborted run.

Deionize the inner surface of each tray and cover:

- The surface of the tray with the wells that hold reagents.
- The surface of the cover that faces the reagents.



CAUTION! Do not deionize the scan tray or hybridization tray.

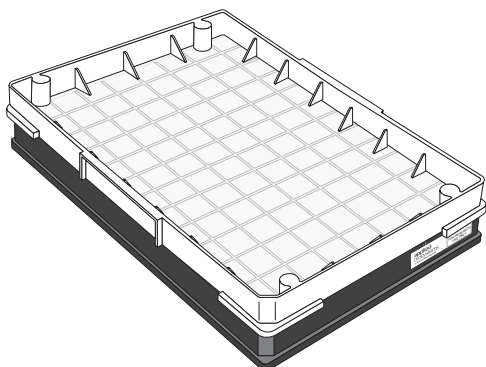


Figure 72 96-format scan tray and cover. Deionize only the cover.

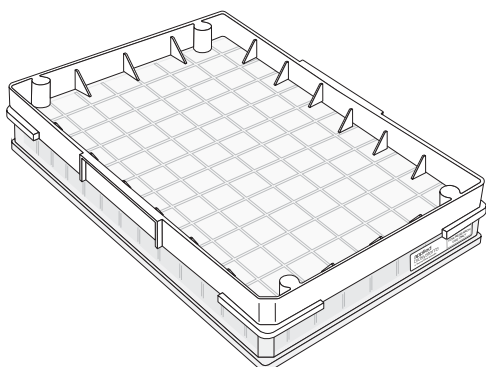


Figure 73 96-format stain tray and cover. Deionize the cover and the tray.

Deionize GeneTitan™ trays and covers



WARNING! The deionization steps damage the arrays on the plate. Before using the antistatic gun, ensure that the array plates remain in their protective pouch and placed away from the deionization area.

Place the scan tray and hybridization tray away from the area where you are performing deionization.

During this procedure, treat the plate or cover as if it were divided into 6 sections. See Figure 74.

1. Place a laboratory tissue on the benchtop.
2. Place the stain tray on a table top. Use the antistatic gun, then squeeze, then release the trigger slowly 3 times over the center of each section, squeezing for approximately 2 seconds, then releasing for approximately 2 seconds.

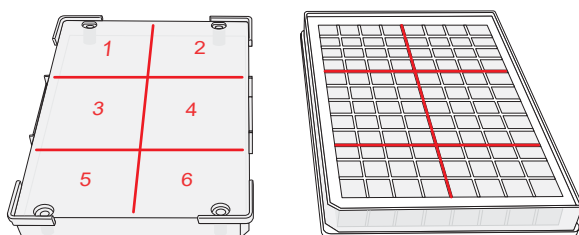
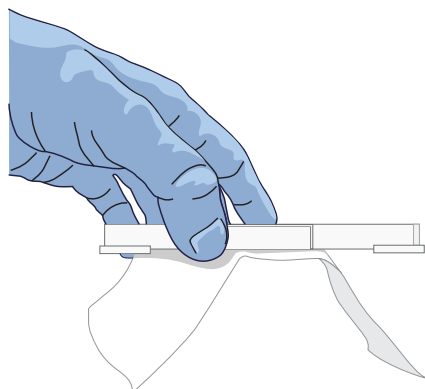


Figure 74 Deionization zones for GeneTitan™ 96-format trays and covers.

Ensure that a stream of ionized particles settles on all wells of the stain tray to dissipate the static electricity.

3. Place the stain tray cover on the tissue with the flat surface facing upward.
4. From ~13 mm away, aim the antistatic gun at the flat surface of the stain tray cover, then squeeze the trigger. As you squeeze the trigger, move the gun across the cover so that the stream of ionized particles settles on all areas of the cover to dissipate the static electricity.
Squeeze and release the trigger slowly 3 times over each section, squeezing for approximately 2 seconds and releasing for approximately 2 seconds.
5. Place the treated cover or tray on the laboratory tissue, then lift it up.



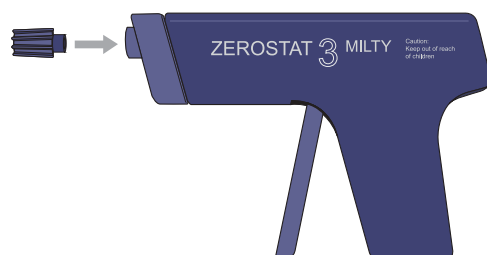
6. Do one of the following:
 - If the tissue does not cling to the plastic, proceed with the protocol.
 - If the tissue clings to the plastic, then repeat step 2 through step 5. If the tissue continues to cling to the plastic, test the device using the ion-indicator cap to determine if the unit is still releasing ions. Otherwise, consider replacing the unit.

Ion-indicator cap

The GeneTitan™ ZeroStat AntiStatic Gun includes an ion-indicator cap. The cap is a device that is used to test the release of ions when the antistatic gun is in use.

Test the antistatic gun for ion release

1. Insert the ion-indicator cap into the nose of the GeneTitan™ ZeroStat AntiStatic Gun.



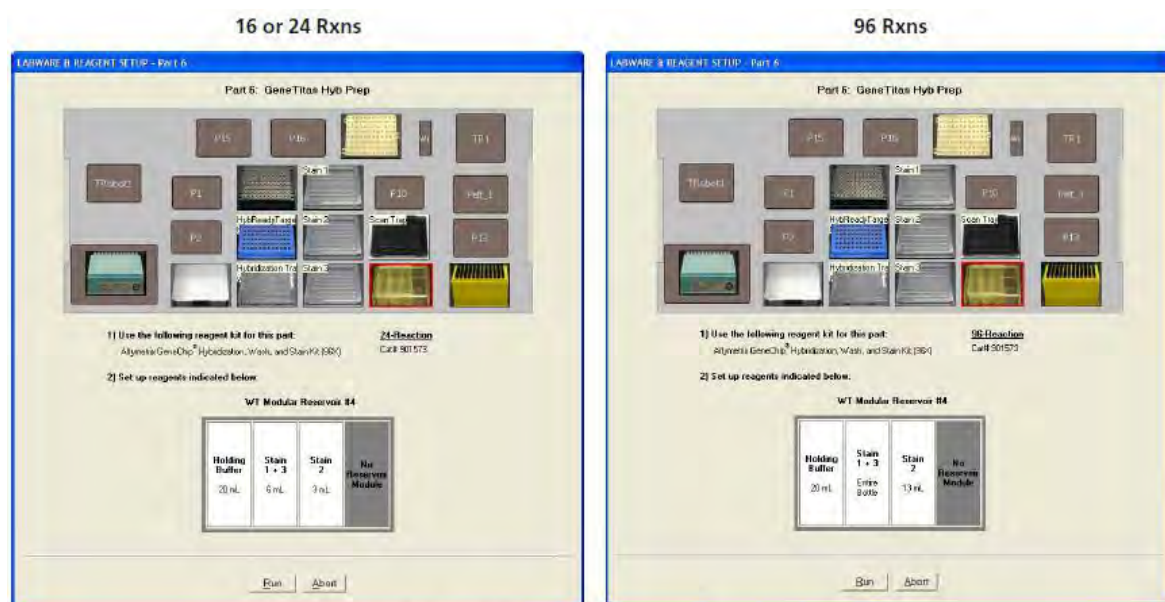
IMPORTANT! Do not leave the ion-indicator cap on the antistatic gun when deionizing trays and covers.

2. Slowly squeeze the release trigger, then observe the discharge through the viewing window on the ion-indicator cap.
 A visible light is observed in the viewing window on the cap when charged ions are discharged.
3. If you cannot see a light through the viewing window, replace the antistatic gun as it is unusable.
 Each GeneTitan™ ZeroStat AntiStatic Gun produces approximately 50,000 trigger operations, which are sufficient for approximately 200-250 runs on the GeneTitan™ MC Instrument.
4. If you can see a light through the viewing window, then have determined that the gun is functional, remove the cap from the gun before deionizing a tray or cover.

Continue the run—Part 6—GeneTitan™ MC Instrument Hybridization Preparation

If you are beginning the run at Part 6, click **Run** below the menubar, then make selections from the **Run Settings Options** window. Click **OK**, then continue with the following instructions.

1. After continuing the run from Part 5 or resuming the run by clicking **Run** and selecting run options, the **Labware & Reagent Setup** window appears (Figure 75).



Please Note: The difference between the screen images is the reagent volumes in the modular reservoir.

Figure 75 Part 6 labware and reagent setup.

Note: If the run was stopped at the end of Part 5, manually place the sample plate at deck position P5 without a lid when ready to resume Part 6.

2. Set up the modular reservoir and place the labware on the deck as directed in the following figures and table:

- Figure 75—Illustration of the deck layout
- Figure 76—Location names of empty deck positions

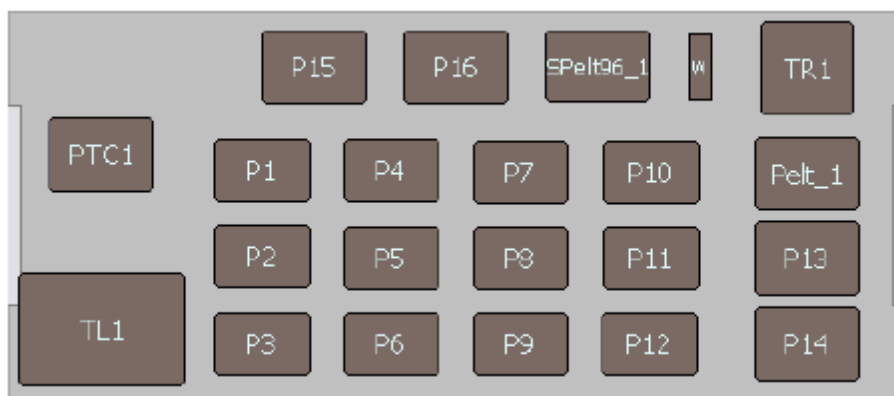


Figure 76 Empty deck positions.

- Table 43—Pipette the correct reagent volumes in the modular reservoir. See Table 43 detailing the labware and Modular Reservoir placement for the deck layout.

Table 43 Labware and reagent locations on the deck for Part 6.

Position on deck ^[1]	Labware		Reagent or samples			
TL1	Biomek™ AP96—P250 Barrier Tips (aqua)	P200—Hyb				
P3	Auto-sealing Microplate Lid with Microseal™ P Pad	Lid				
P4	Alpaqua™ Magnetic Plate	Magnetic Plate				
P5	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate	Hybridization-ready Target	Hybridization-ready target from Part 5			
P6	GeneTitan™ Hybridization Tray	Hybridization Tray				
P7	GeneTitan™ Stain Tray	Stain 1				
P8	GeneTitan™ Stain Tray	Stain 2				
P9	GeneTitan™ Stain Tray	Stain 3				
P11	GeneTitan™ Scan Tray	Scan Tray				
P12	Reservoirs in frame:	Reagent Reservoirs	1	2	3	4

Table 43 Labware and reagent locations on the deck for Part 6. (continued)

Position on deck ^[1]	Labware		Reagent or samples			
P12	- Quarter module reservoir in position 1, 2 and 3 - Position 4 is empty - Pipette Holding Buffer into reservoir 1 16 reactions = 20 mL 24 reactions = 20 mL 96 reactions = 20 mL - Pipette Stain 1 & 3 into reservoir 2 16 reactions = 6 mL 24 reactions = 6 mL 96 reactions = entire bottle - Pipette Stain 2 into reservoir 3 16 reactions = 3 mL 24 reactions = 3 mL 96 reactions = 13 mL	Reagent Reservoirs	Holding Buffer	Stain 1 & 3	Stain 2	Empty
P14	Biomek™ Span P1000 LLS Barrier Tips (yellow)	P1000—Span8				
Spelt96_1	Peltier Adapter (installed on deck)					

^[1] See Figure 76 for empty deck positions.

- When ready to continue, click **Run** in the **Labware & Reagent Setup** window (Figure 75).
- In the prompt that appears, click **Yes** to begin purging the Pod2 syringe lines.

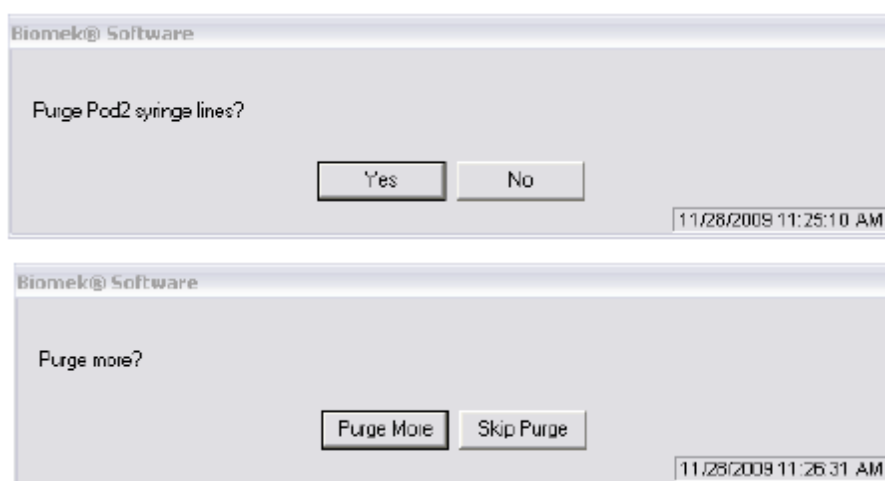


Figure 77 Prompts to purge Pod2 syringe lines.

If bubbles are still present after purging, click **Purge More** in the next prompt (Figure 77). If no further purging is required, click **Skip Purge**. The run proceeds.

At the completion of Part 6, the message shown in Figure 78 appears. The HT WT Expression Assay for array plates is now complete.



Figure 78 Message at completion of Part 6.

5. Follow the instructions in the message box (Figure 78)
 - a. Discard the labware listed.
 - b. Remove and save the following:
 - Magnetic Plate
 - Modular Reservoir Rack
 - Tube Rack
 - Span-8 tips
 - Sample Plate

c. Prepare for the GeneTitan™ MC Instrument .

1. Examine each tray to ensure that:
 - All of the wells as appropriate (24 or 96) have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
 - There are no air bubbles present. Puncture any air bubbles that you see using a pipette tip.
2. Carefully remove the following plates and transfer to the GeneTitan™ MC Instrument :
 - Hybridization tray with target
 - Cover and remove stain trays with stains
 - Cover and remove scan tray with array holding buffer

IMPORTANT! Immediately load the reagent plates onto the GeneTitan™ MC Instrument . Then load the array plate and hybridization tray. Do not leave samples or reagent plates at room temperature for any length of time.

6. Proceed to processing array plates using the GeneTitan™ MC Instrument according instructions in the *GeneTitan™ Instrument User Guide for Expression Array Plates*.



Using the 24-reaction kit for two 8-sample runs

This appendix provides instructions for using the Invitrogen™ WT Expression Kit, high throughput, 24-sample reaction kit (Cat. No. [4440537](#)) for two 8-sample runs.

1. Remove the following enzyme tubes and keep them on ice.
 - First Strand Enzyme Mix
 - Second Strand Enzyme Mix
 - IVT Enzyme Mix
 - Second Cycle Enzyme Mix
 - RNase H
2. Centrifuge the tubes in a microfuge for 5 seconds, then place them on ice.
3. Remove an aliquot from each of the tubes and put it in a new tube (already labeled and put on ice) using the information from the following table.

Reagent	Tube size	Thomas Scientific™ tube Part No.	Volume to be aliquoted
First Strand Enzyme Mix	0.5 mL	649050	25 µL
Second Strand Enzyme Mix	0.5 mL	649050	88 µL
IVT Enzyme Mix	0.5 mL	649050	105 µL
Second Cycle Enzyme Mix	1.5 mL skirted	649010	133 µL
RNase H	0.5 mL	649050	50 µL

4. Centrifuge the tubes in a microfuge for 5 seconds and place them on ice. Use this aliquot for the first 8-sample run.

Note: Before centrifuging, use the lids from the original tubes to cover the newly aliquoted tubes.

5. Store the original tubes at –20°C and use these for the second 8-sample run.
6. Make sure to store the buffer mix tubes at –20°C after the first run.



Workflow diagrams

■ First-strand cDNA synthesis	129
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This appendix provides workflow diagrams depicting the GeneChip™ HT WT Expression Assay Automated Workflow on the Biomek™ FX^P Automated Workstation.

IMPORTANT! The screen images show the blue Bio-Rad™ 96-well PCR plate (HSP9631). This blue plate can be substituted with the white Bio-Rad™ 96-well PCR plate (HSP9601).

First-strand cDNA synthesis

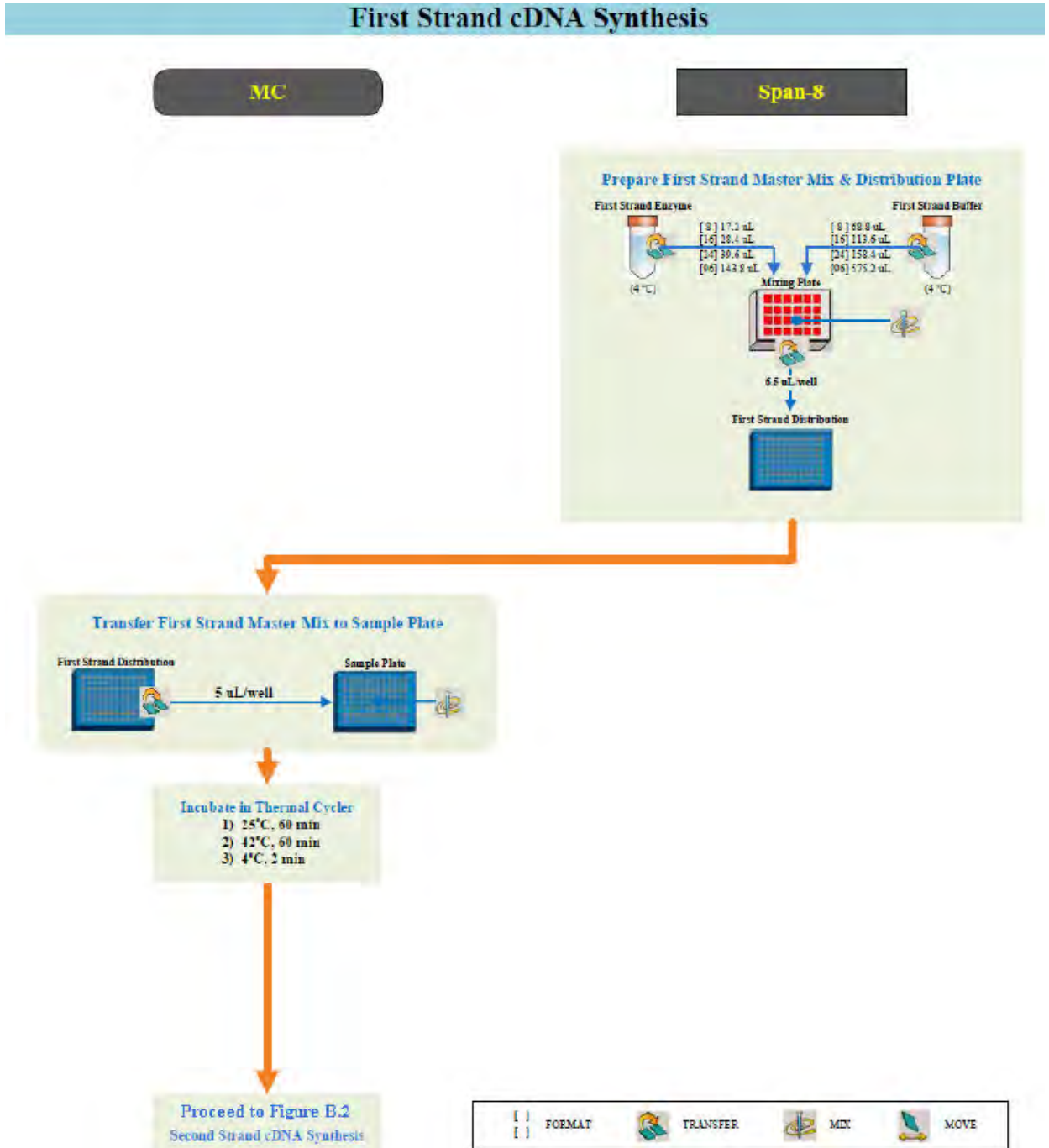


Figure 79 First-strand cDNA synthesis.

Second-strand cDNA synthesis

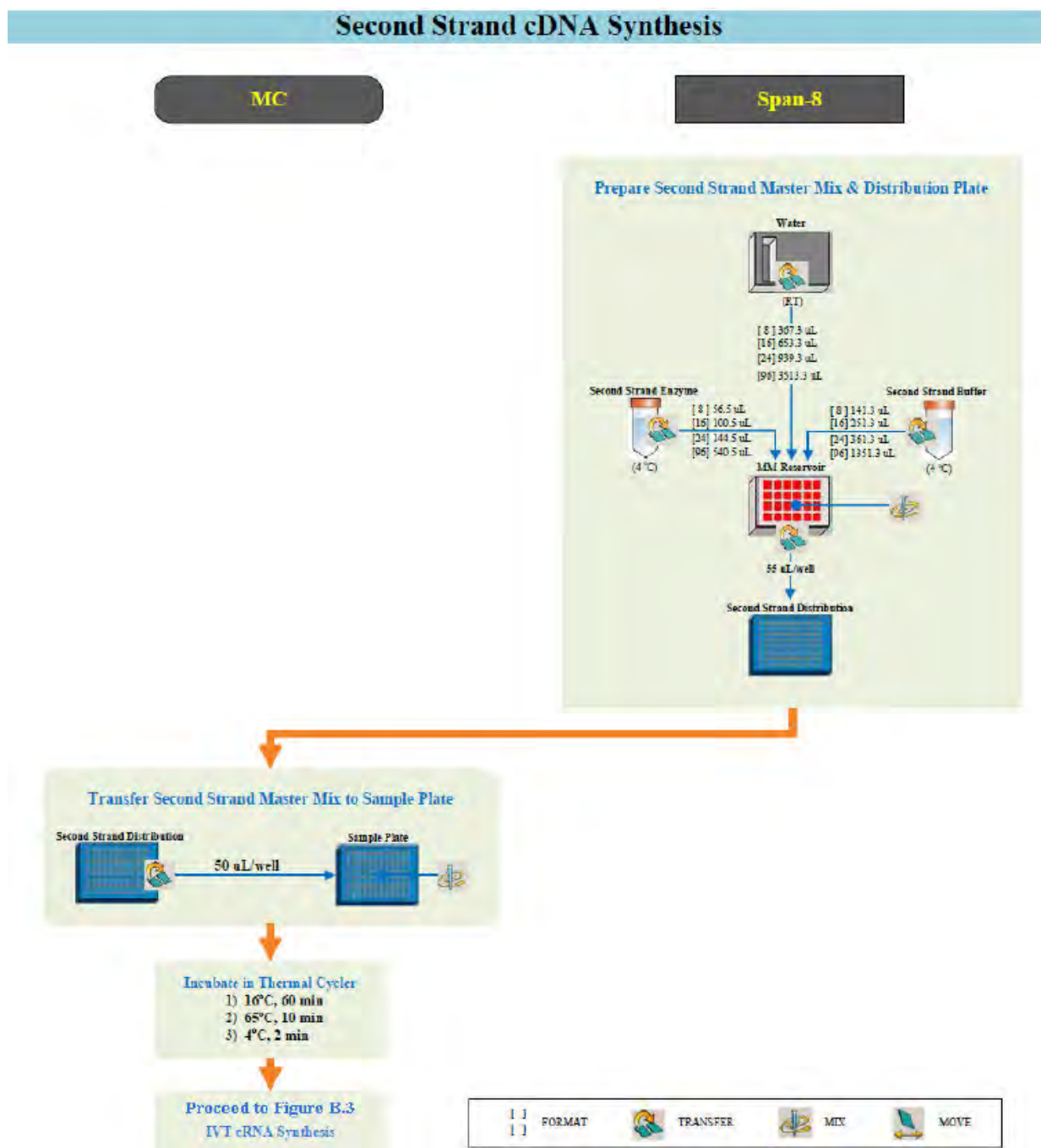


Figure 80 Second-strand cDNA synthesis.

In vitro cRNA synthesis

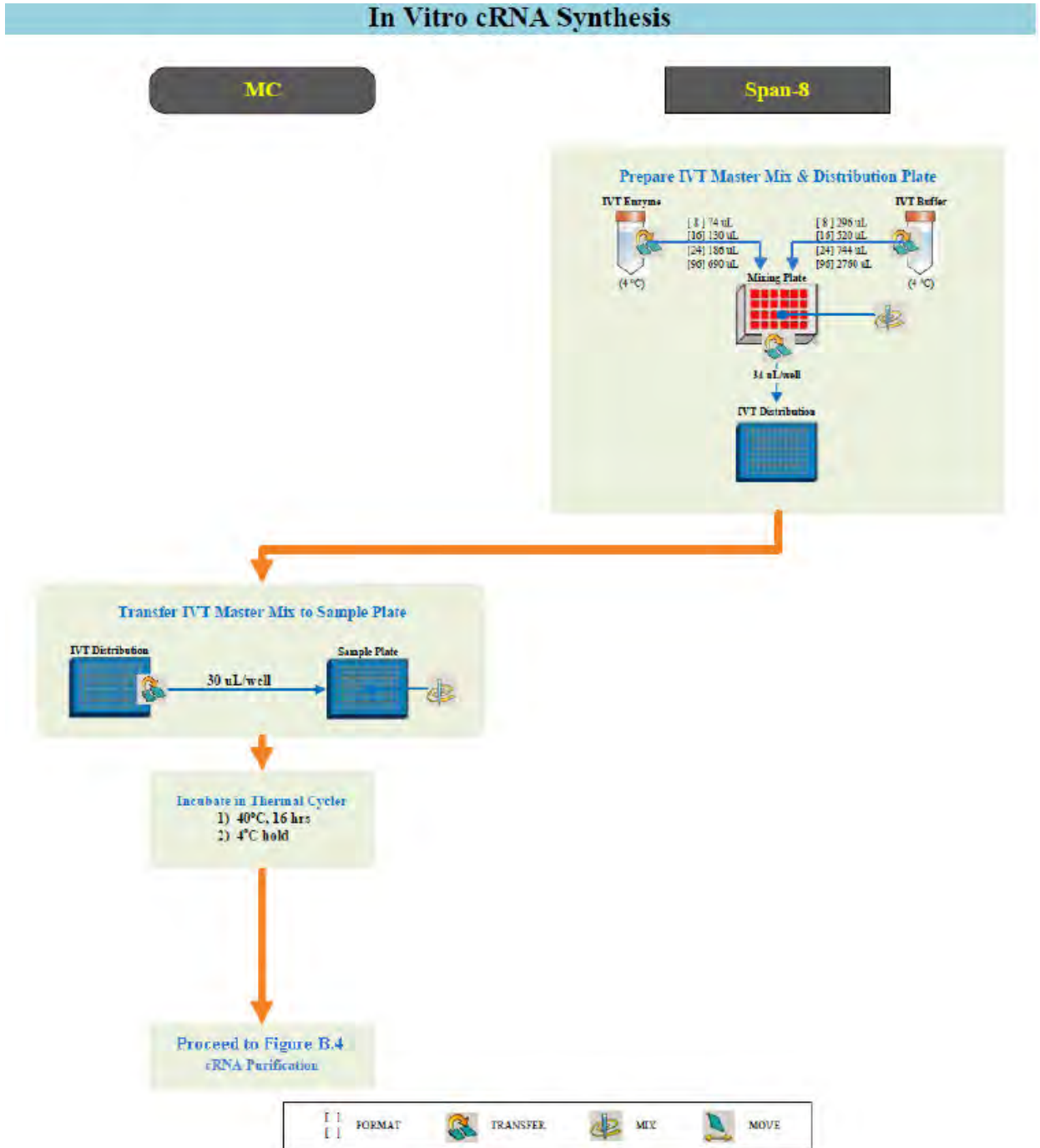


Figure 81 In vitro cRNA synthesis.

cRNA purification

cRNA purification (diagram 1 of 8)

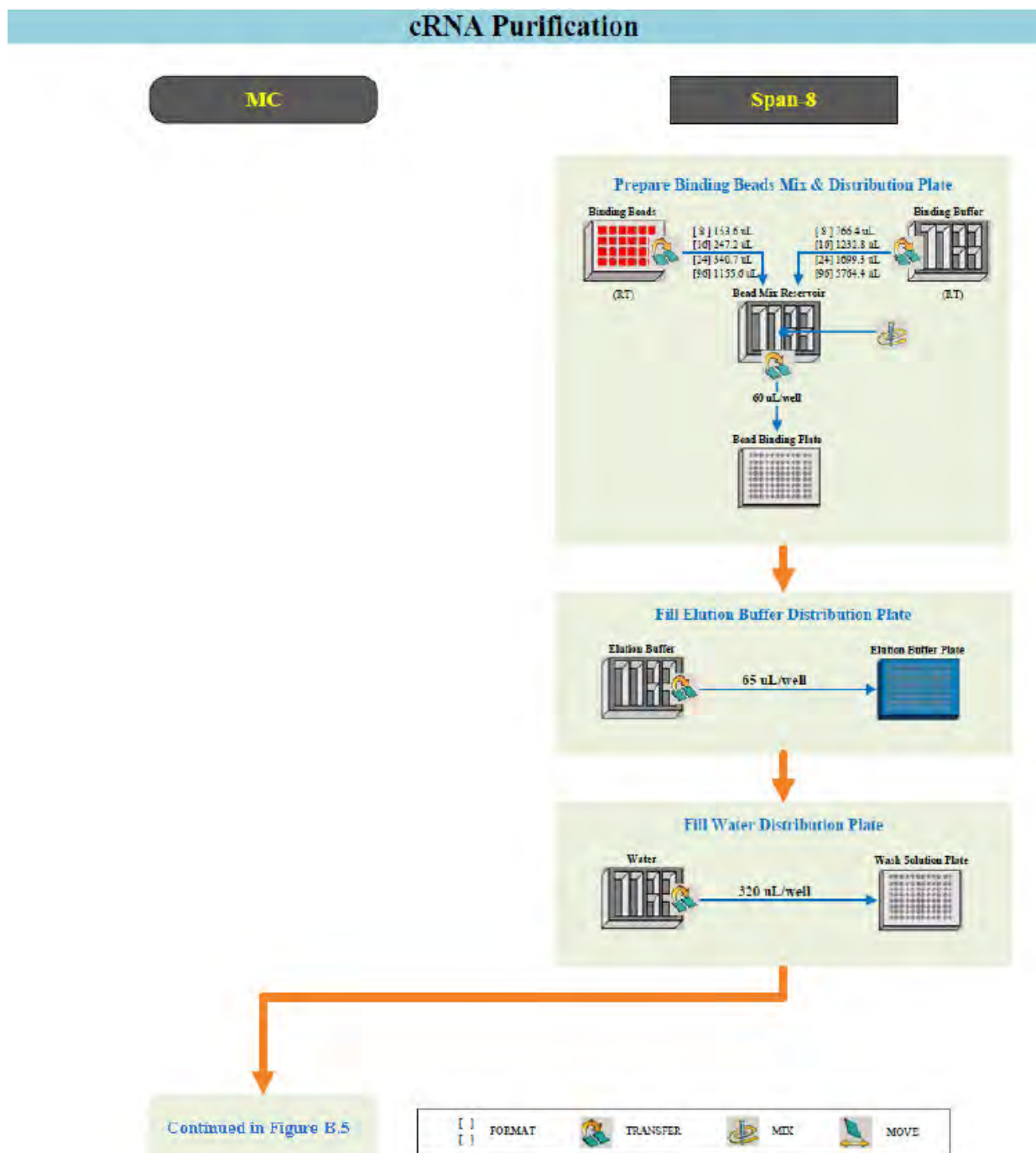


Figure 82 cRNA purification (1 of 8).

cRNA purification (diagram 2 of 8)

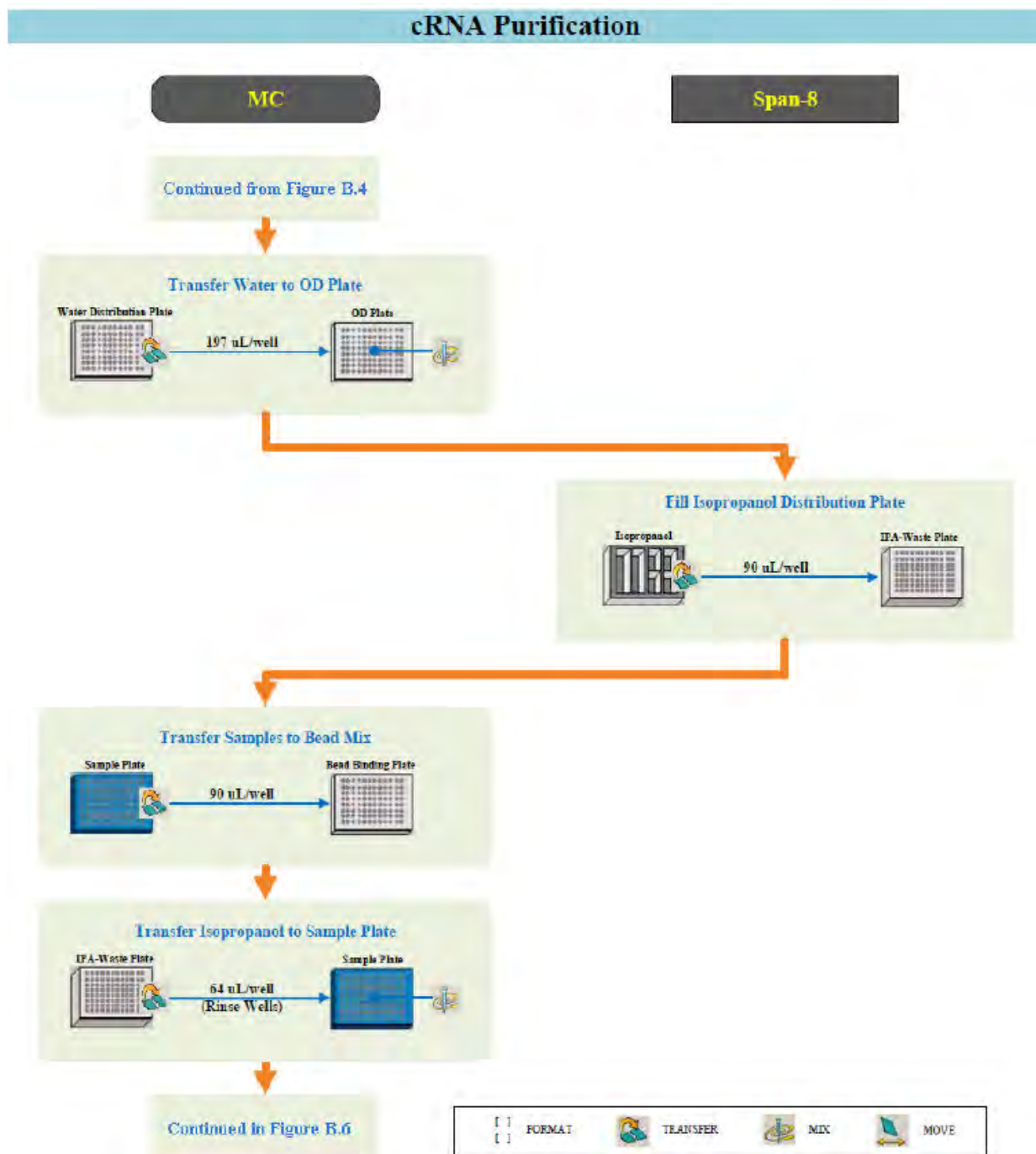


Figure 83 cRNA purification (2 of 8).

cRNA purification (diagram 3 of 8)

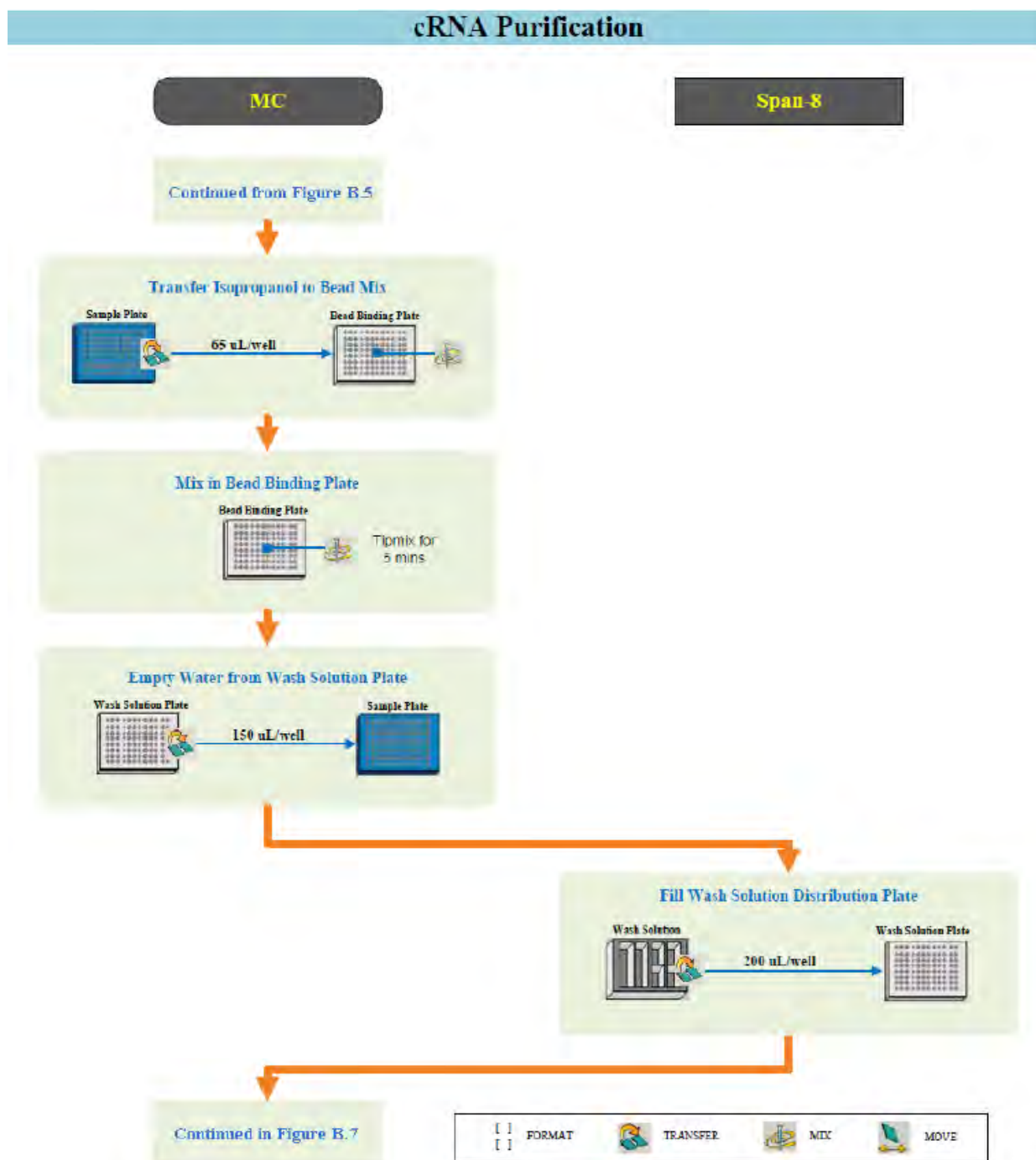


Figure 84 cRNA purification (3 of 8).

cRNA purification (diagram 4 of 8)

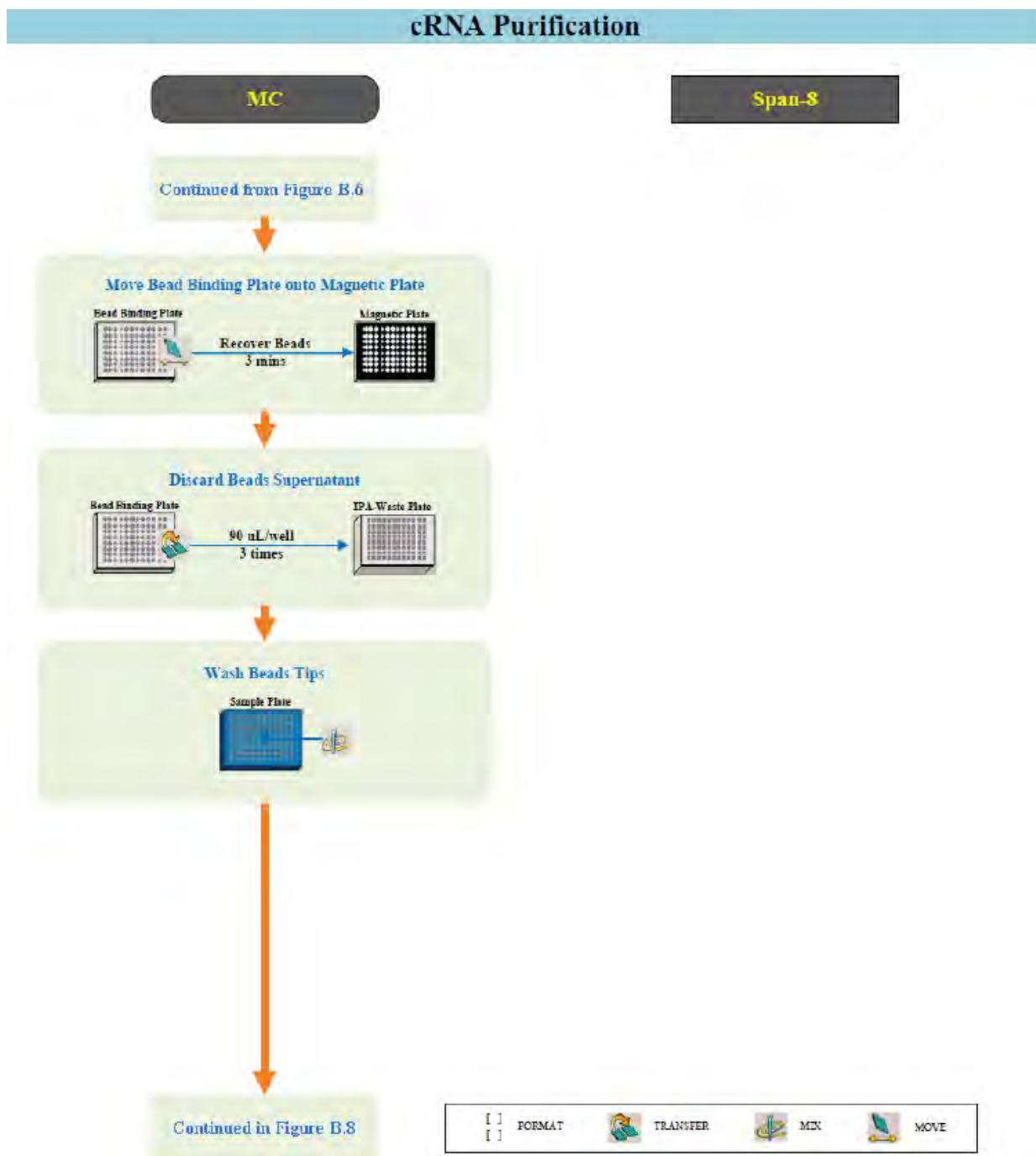


Figure 85 cRNA purification (4 of 8).

cRNA purification (diagram 5 of 8)

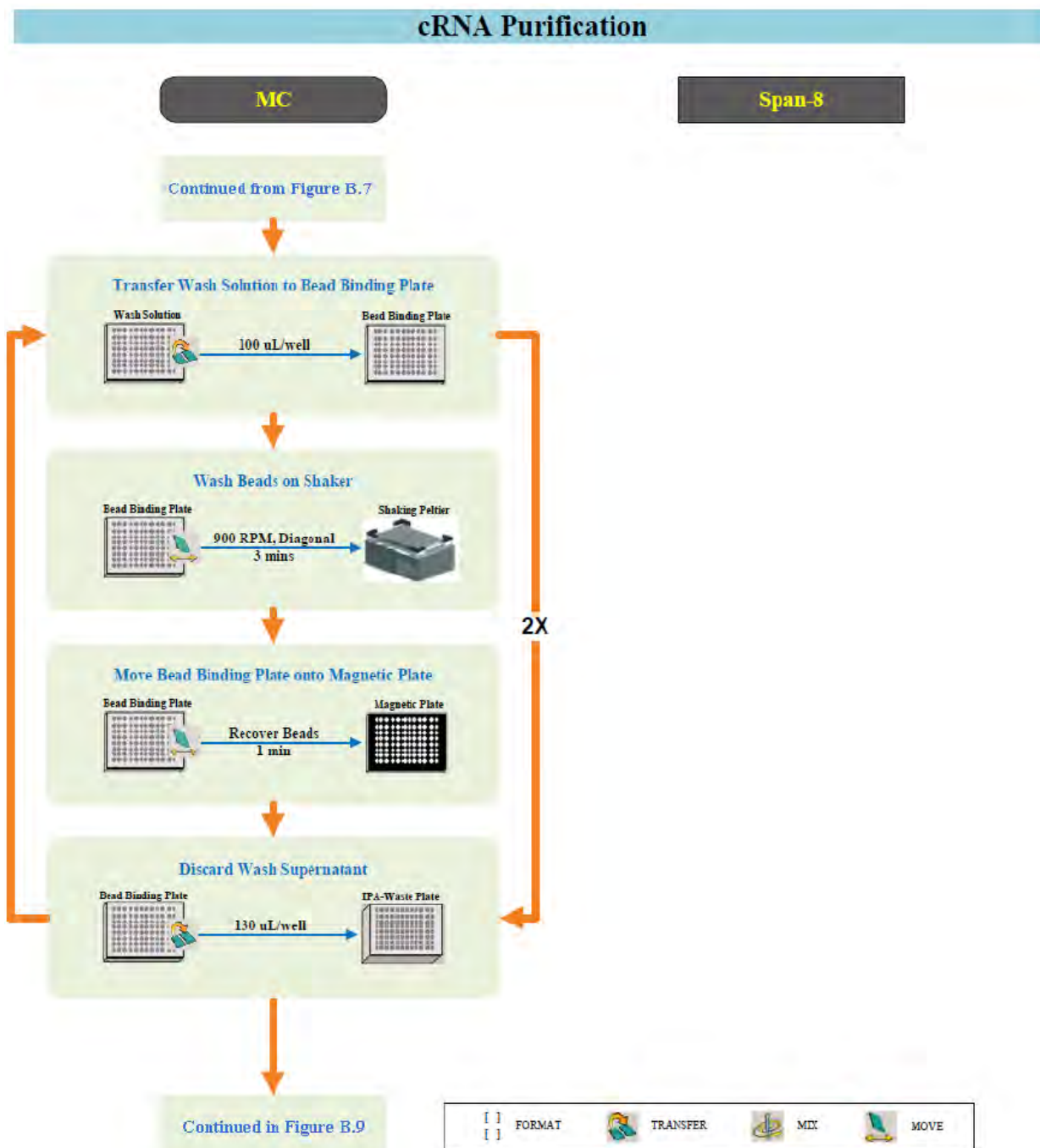


Figure 86 cRNA purification (5 of 8).

cRNA purification (diagram 6 of 8)

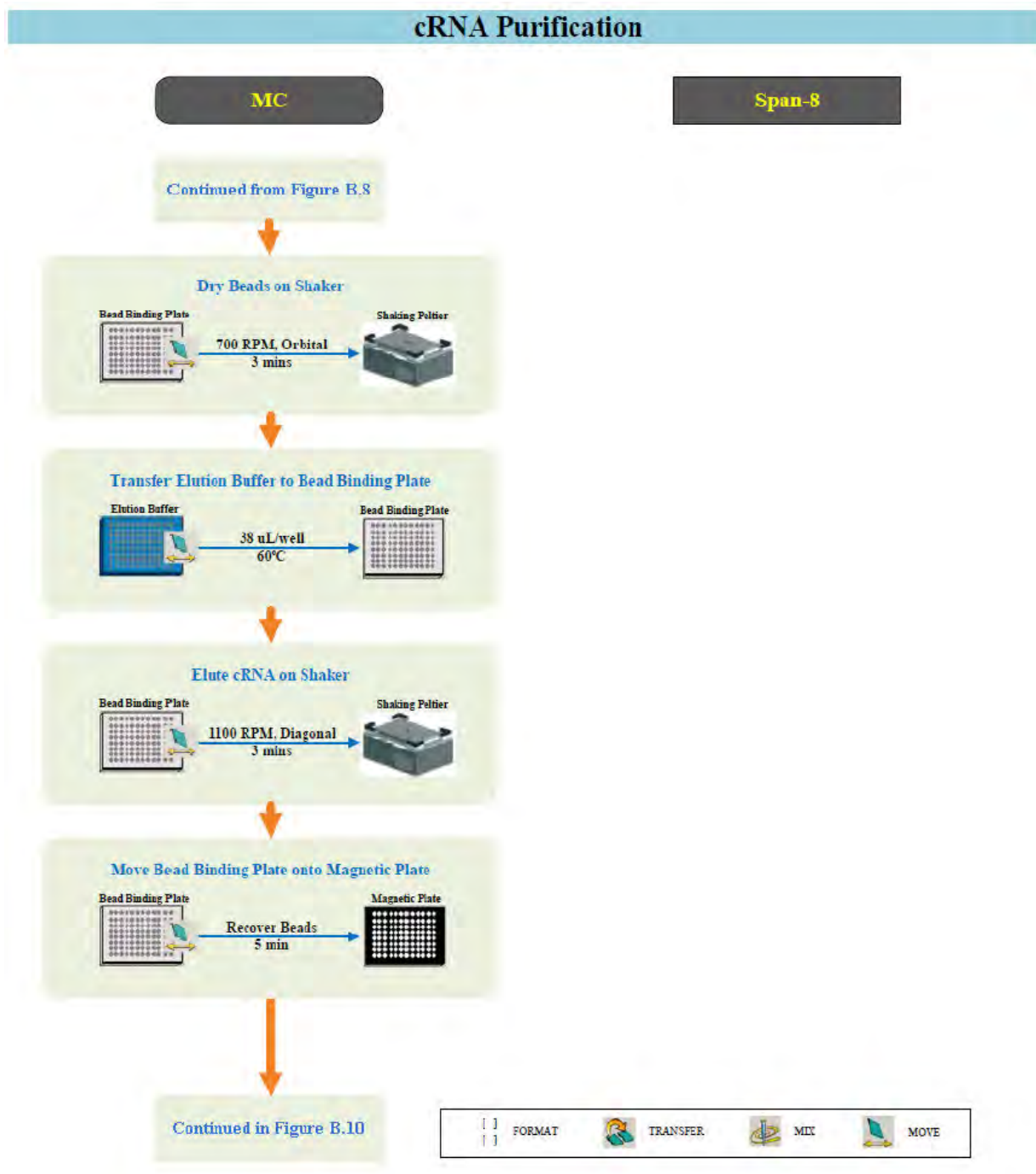


Figure 87 cRNA purification (6 of 8).

cRNA purification (diagram 7 of 8)

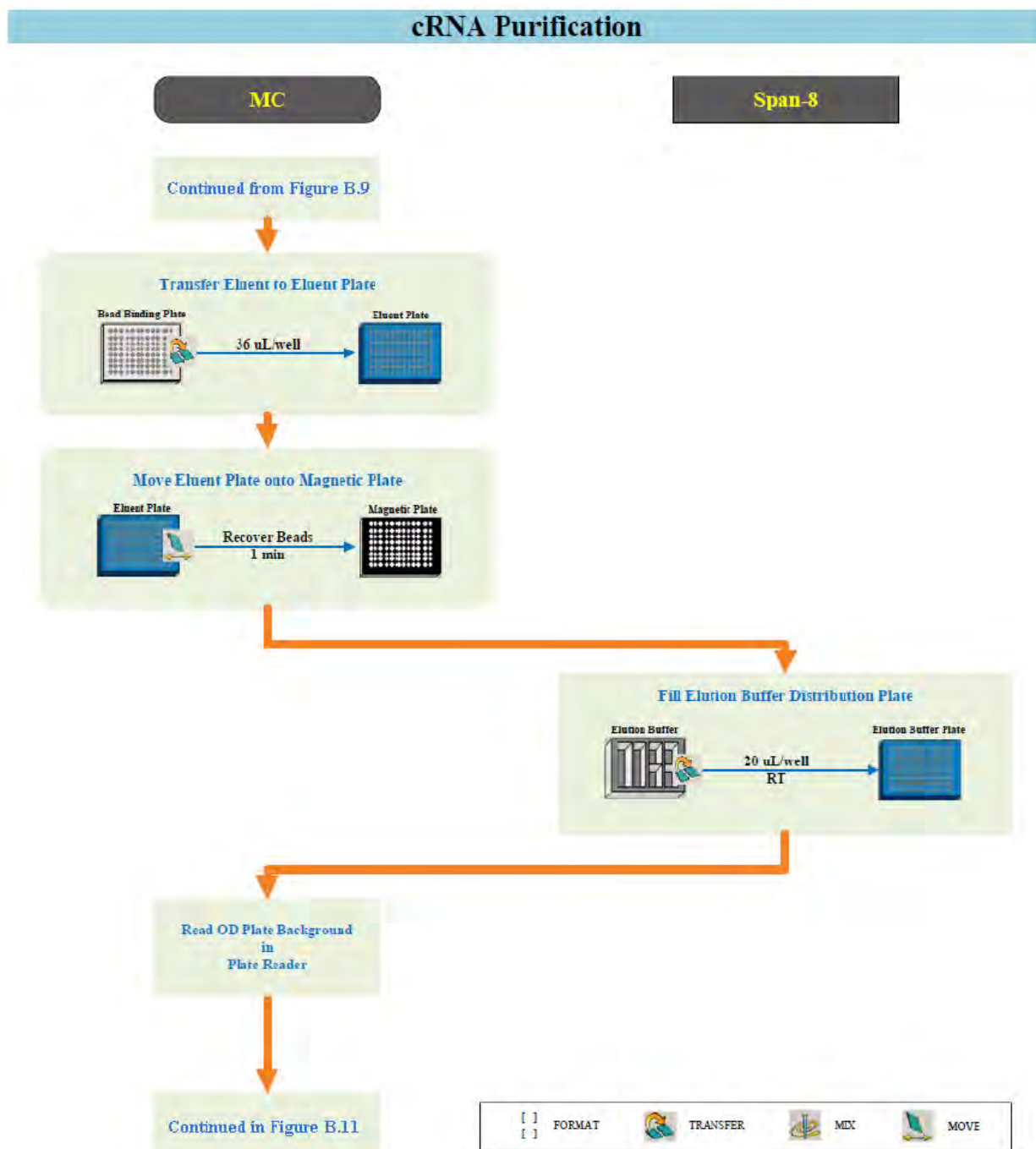


Figure 88 cRNA purification (7 of 8).

cRNA purification (diagram 8 of 8)

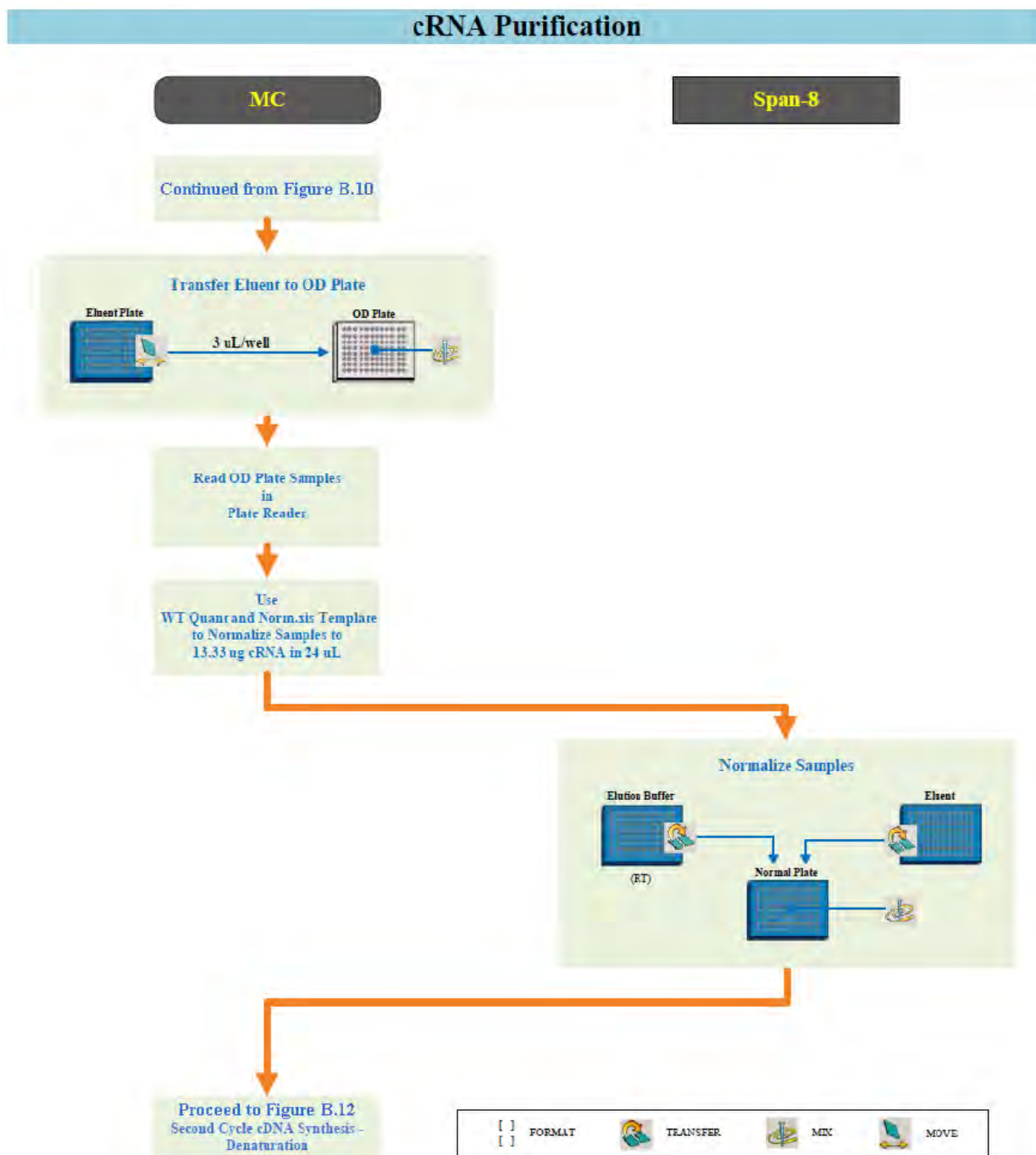


Figure 89 cRNA purification (8 of 8).

Second-cycle cDNA synthesis—denaturation

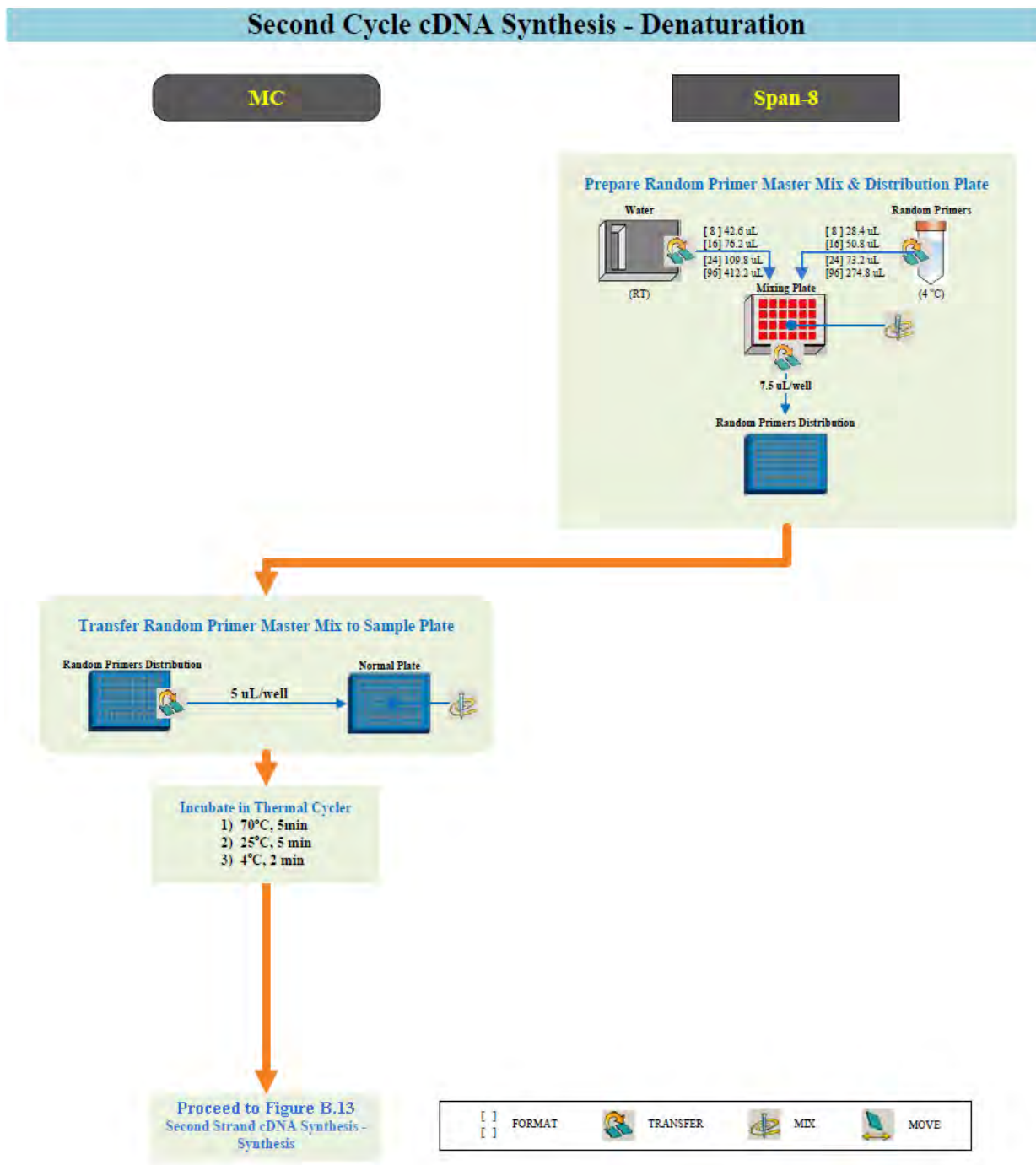


Figure 90 Second-cycle cDNA synthesis—denaturation.

Second-cycle cDNA synthesis—synthesis

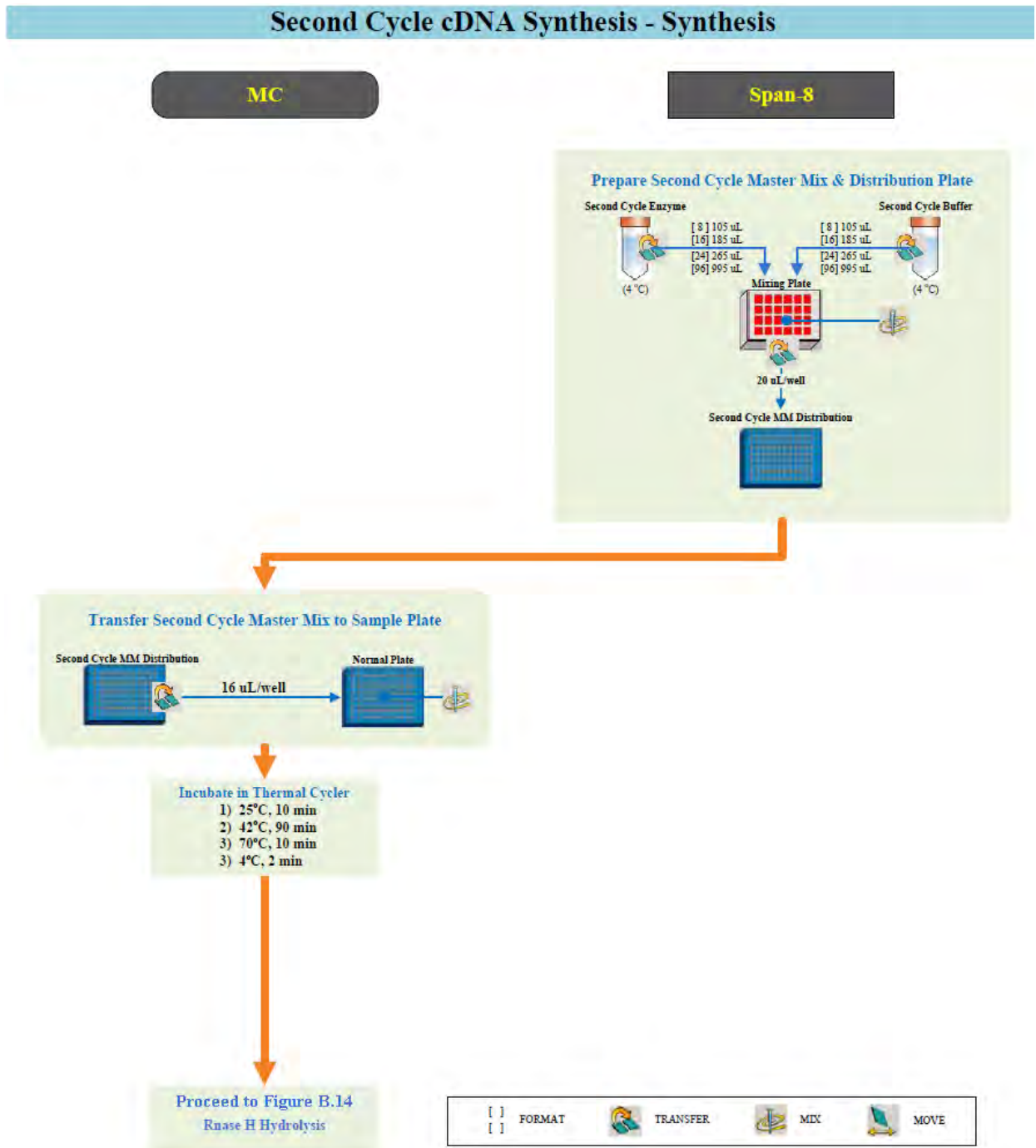


Figure 91 Second-cycle cDNA synthesis—synthesis.

RNase H hydrolysis

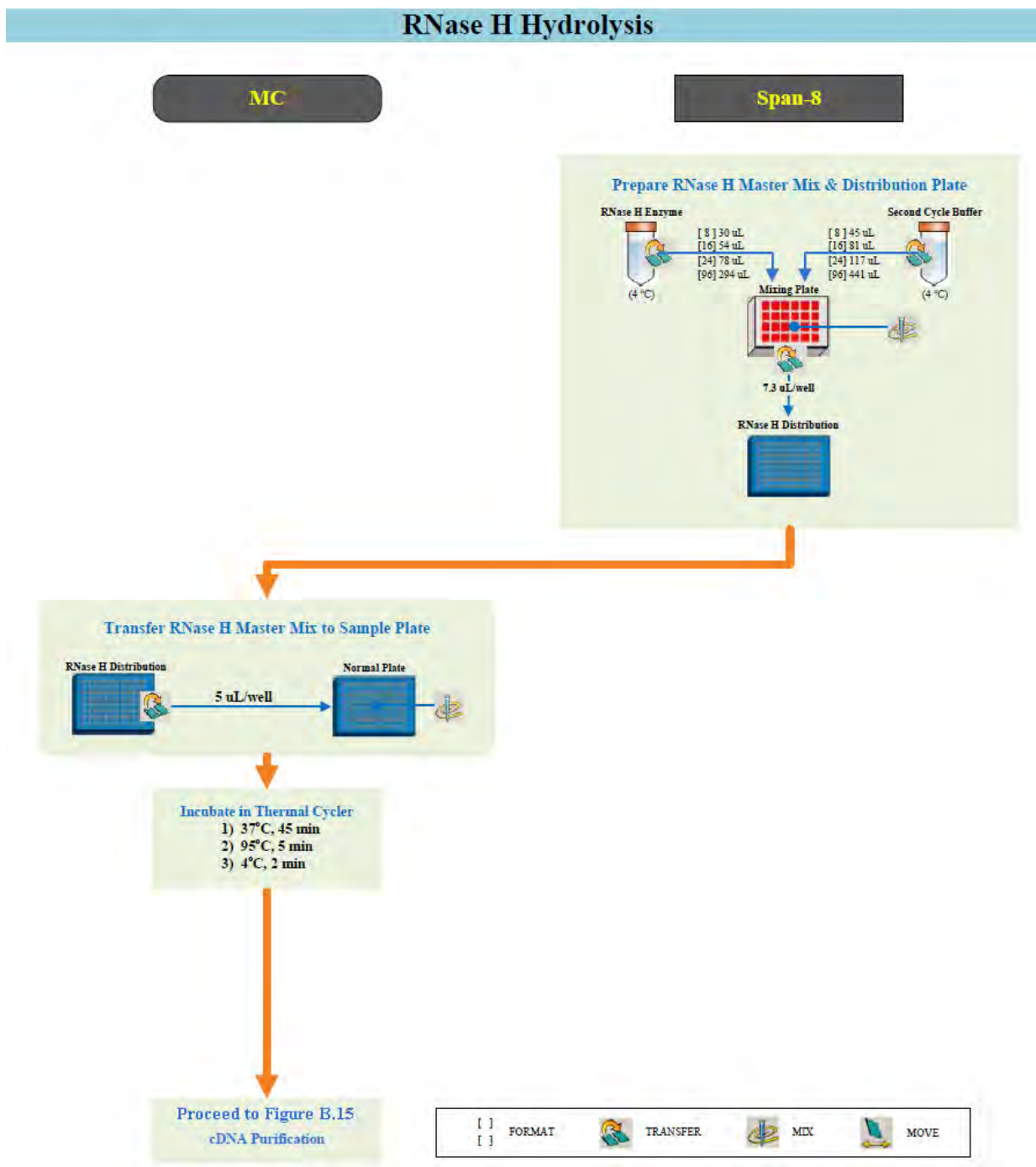


Figure 92 RNase H hydrolysis.

cDNA purification

cDNA purification (diagram 1 of 8)

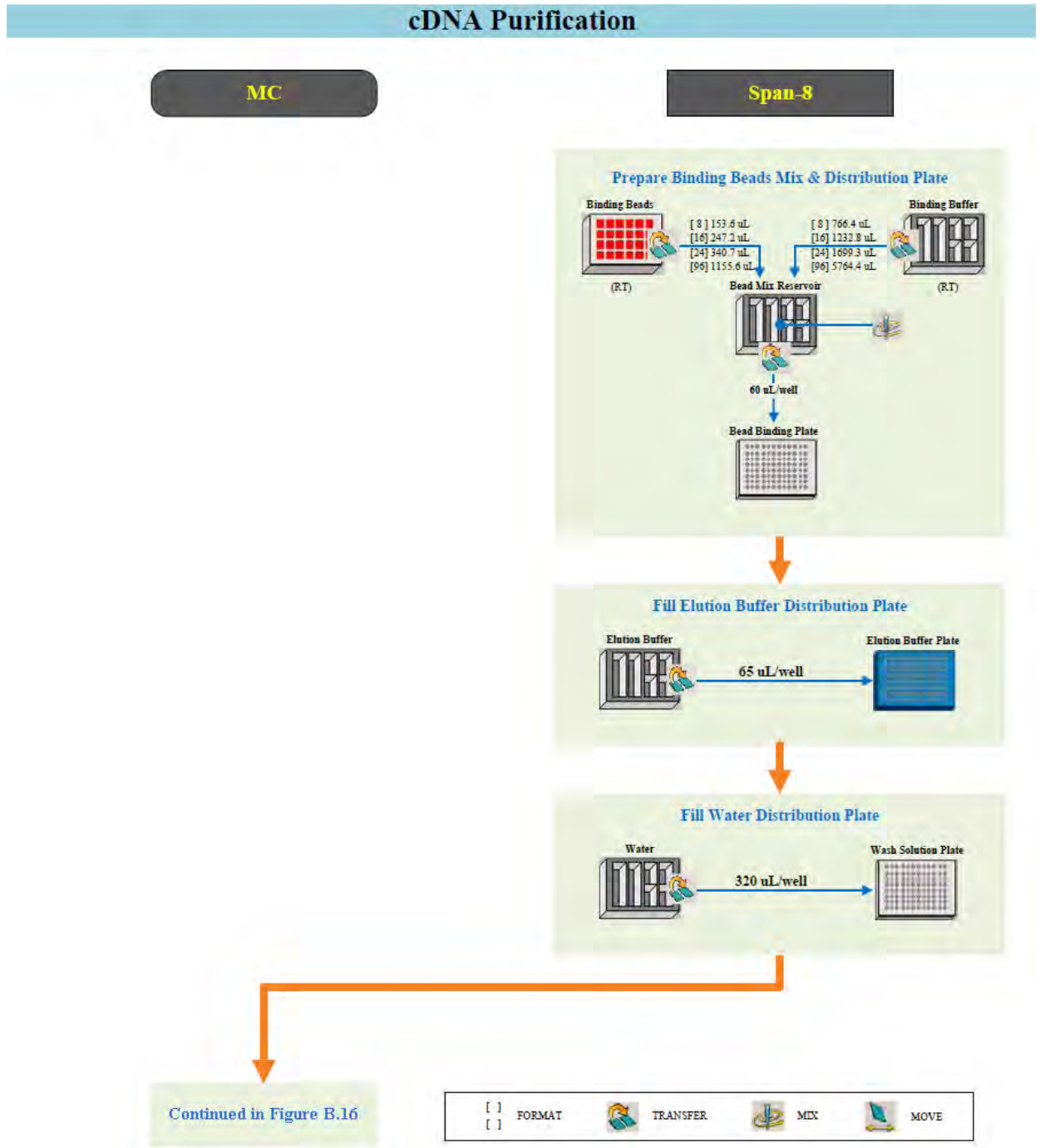


Figure 93 cDNA purification (1 of 8).

cDNA purification (diagram 2 of 8)

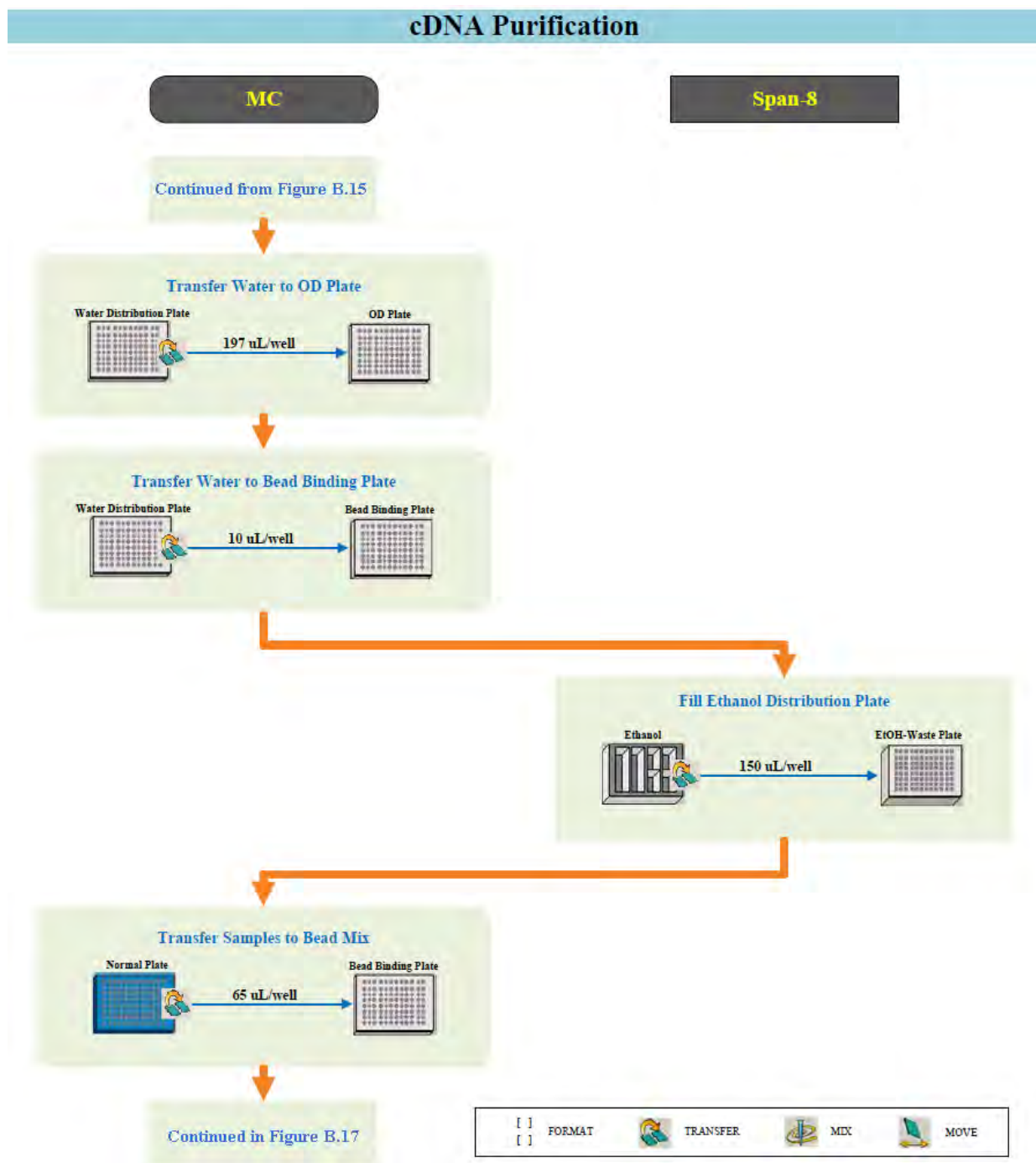


Figure 94 cDNA purification (2 of 8).

cDNA purification (diagram 3 of 8)

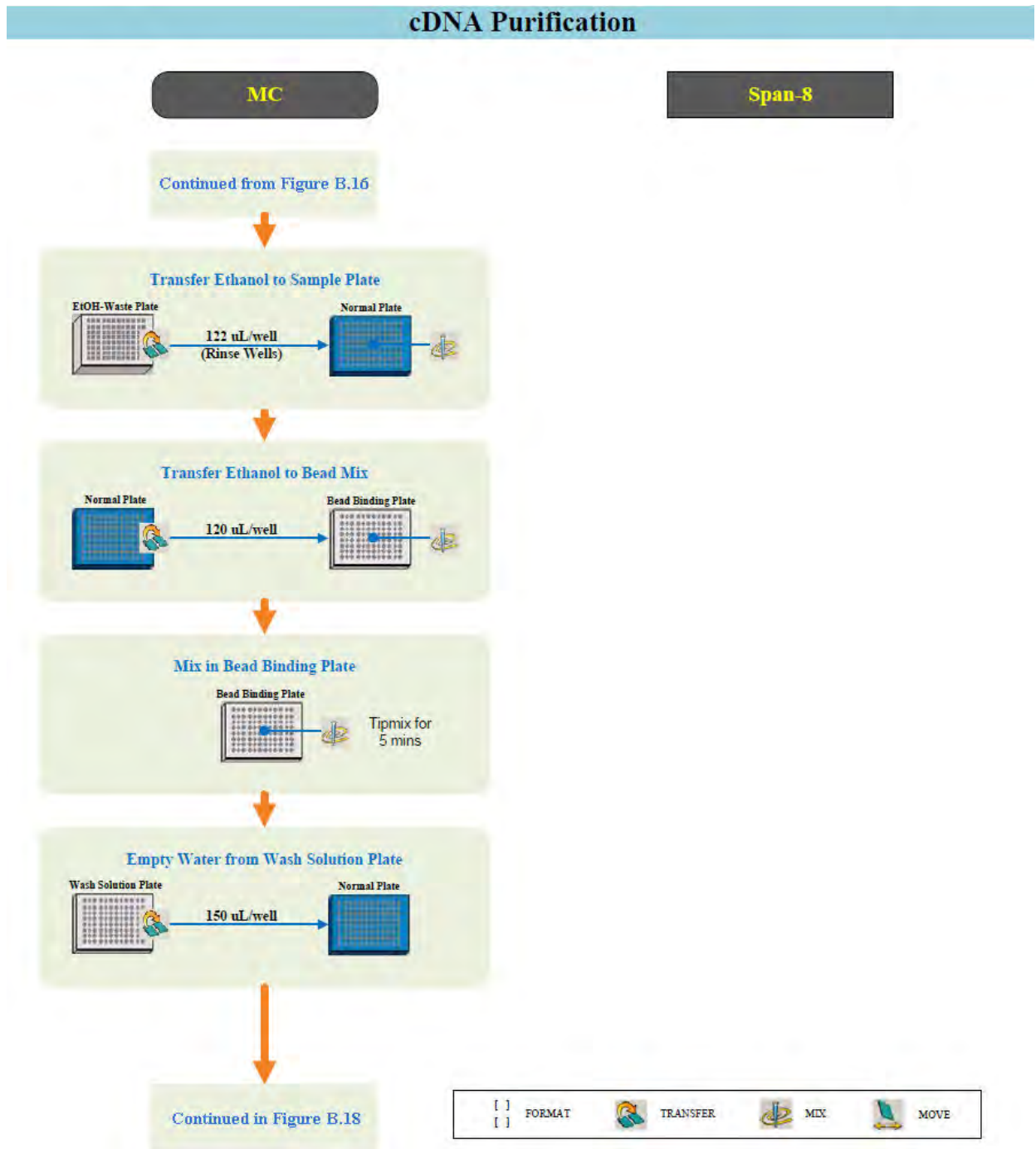


Figure 95 cDNA purification (3 of 8).

cDNA purification (diagram 4 of 8)

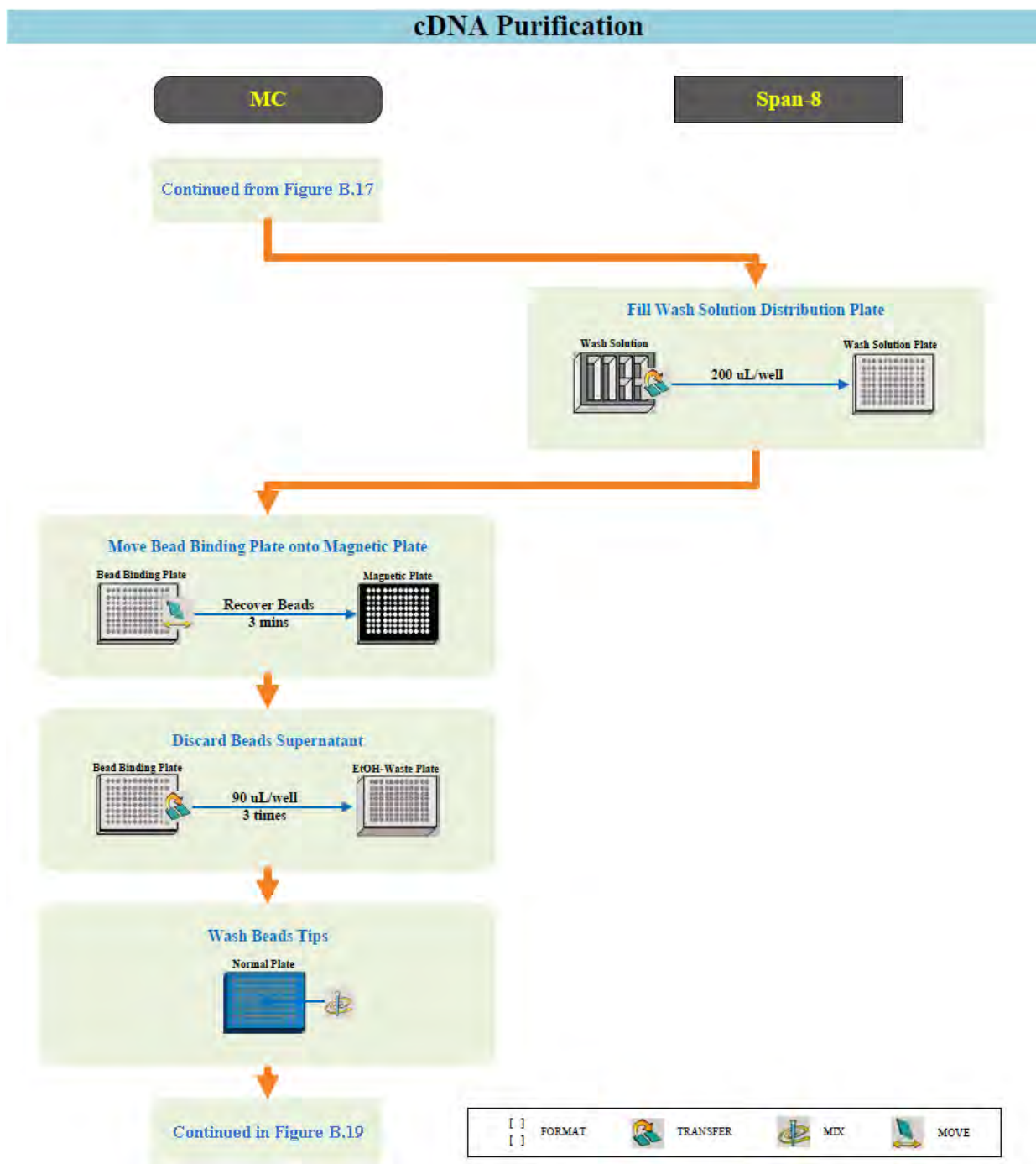


Figure 96 cDNA purification (4 of 8).

cDNA purification (diagram 5 of 8)

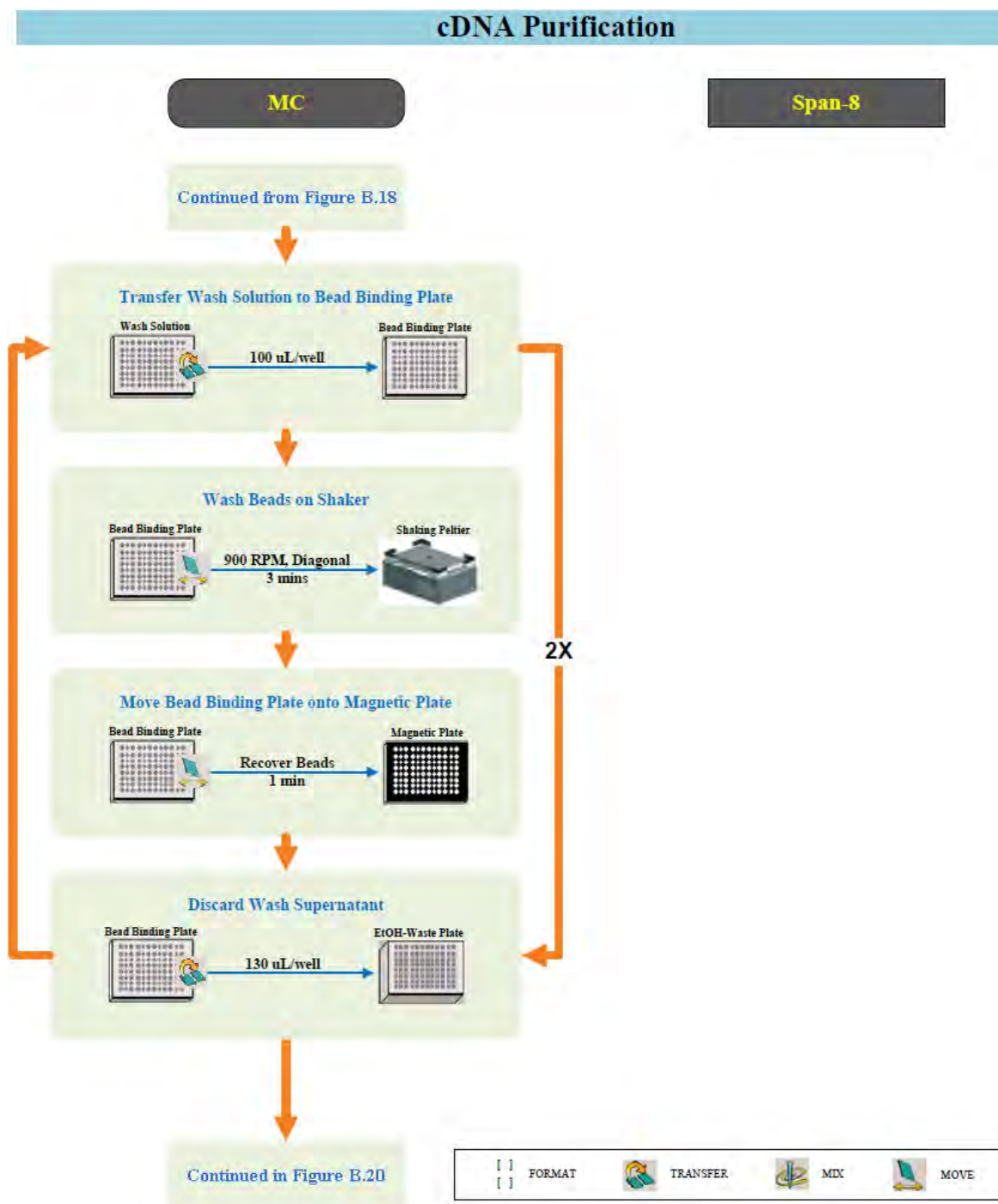


Figure 97 cDNA purification (5 of 8).

cDNA purification (diagram 6 of 8)

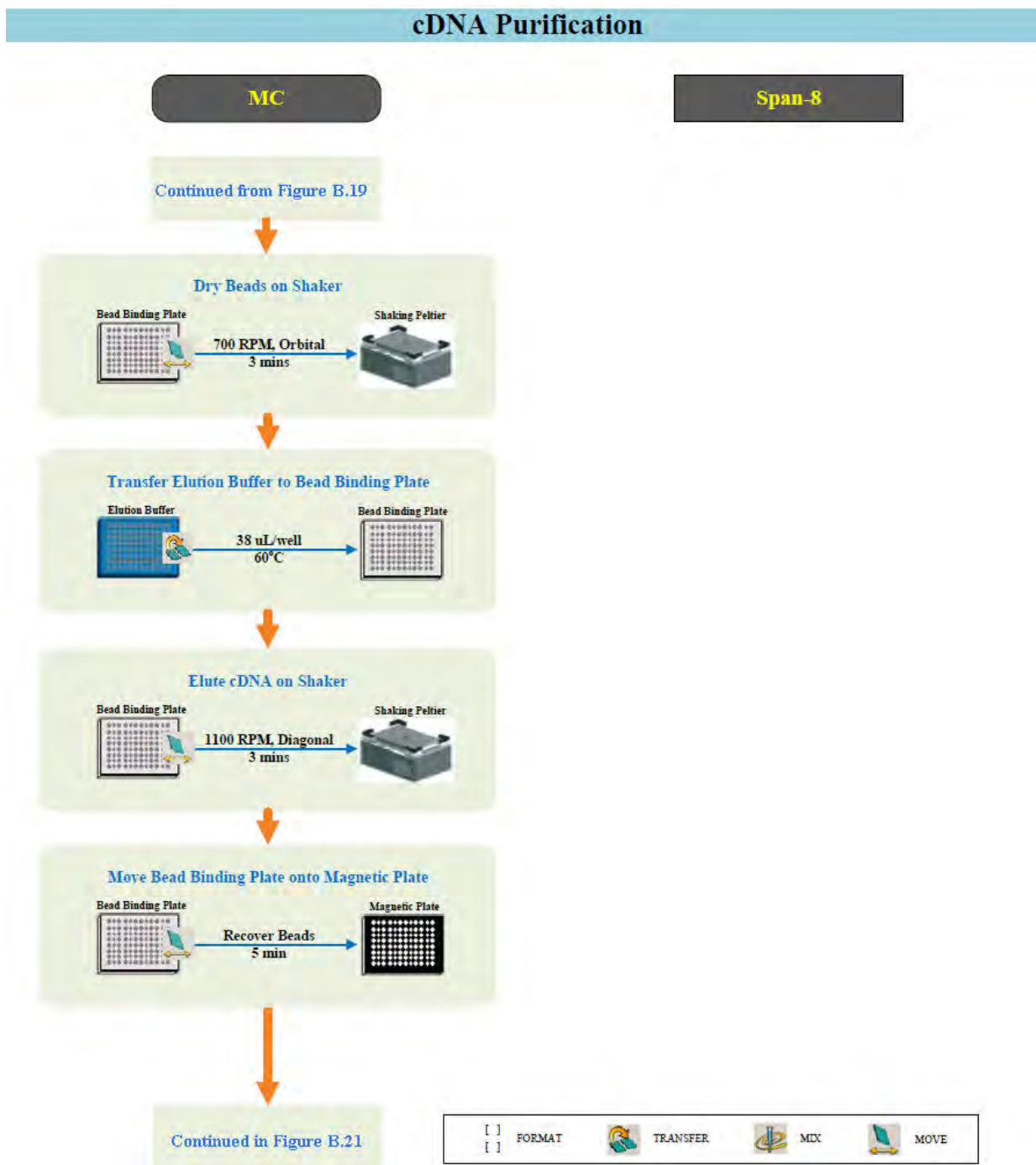


Figure 98 cDNA purification (6 of 8).

cDNA purification (diagram 7 of 8)

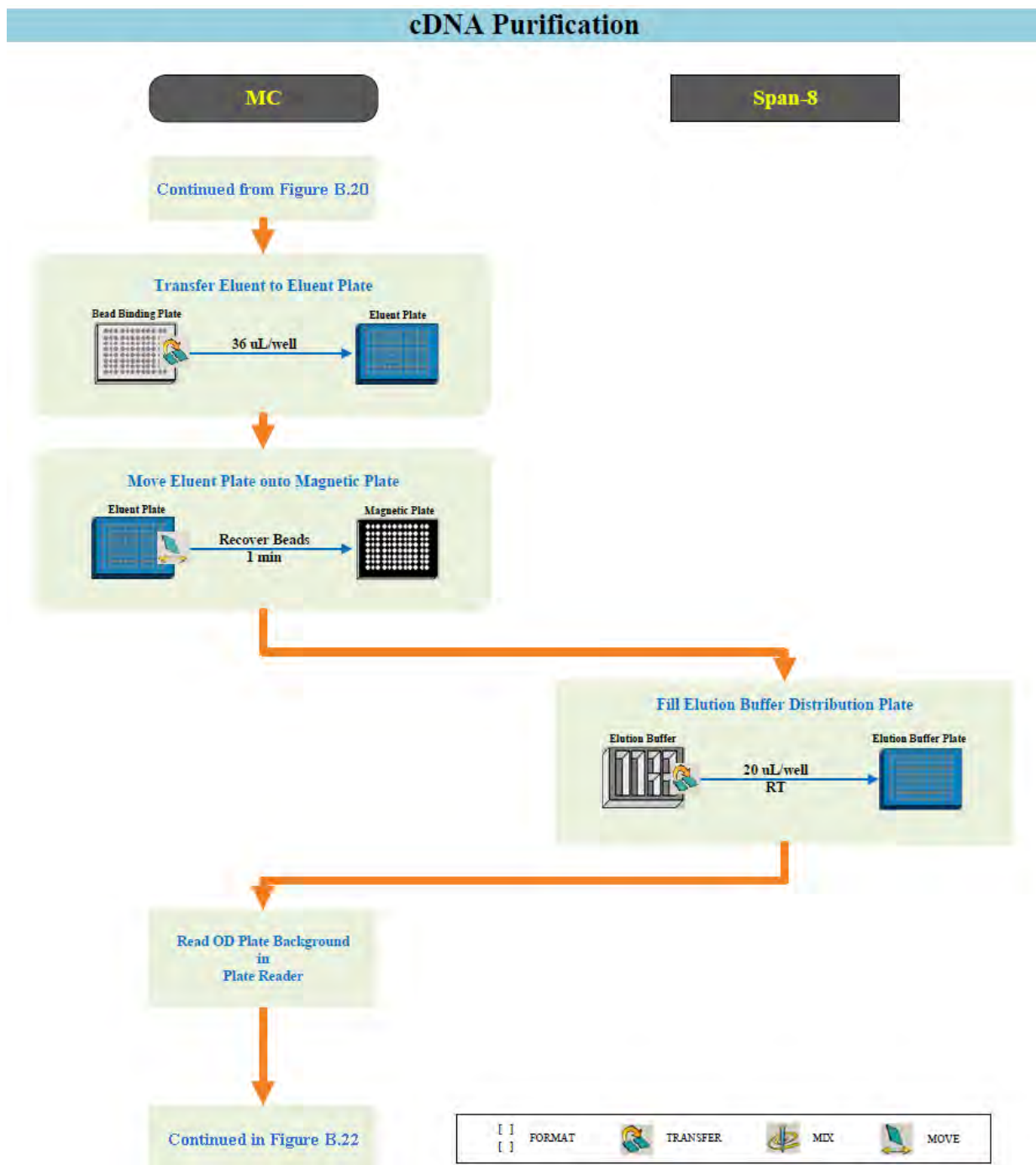


Figure 99 cDNA purification (7 of 8).

cDNA purification (diagram 8 of 8)

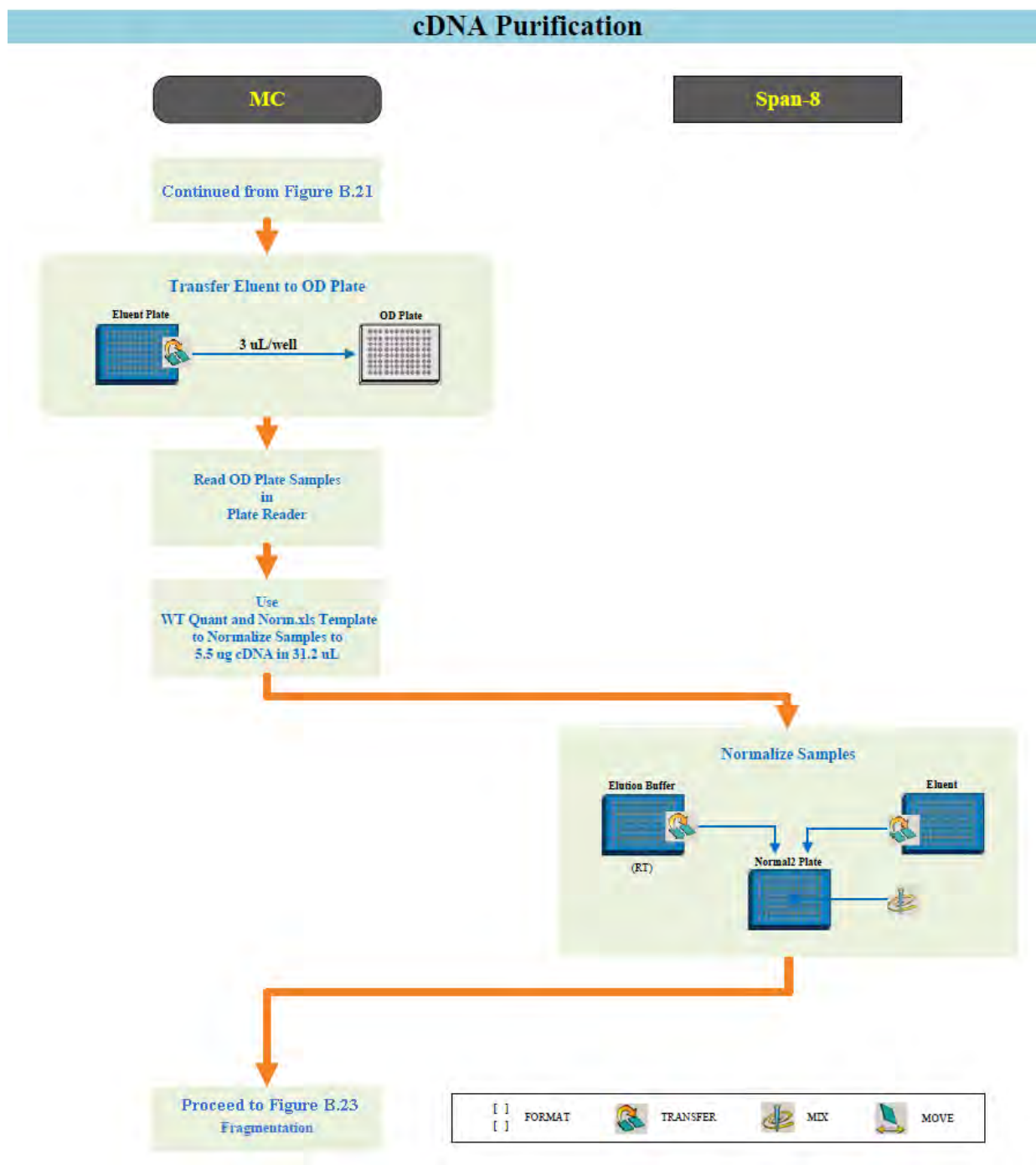


Figure 100 cDNA purification (8 of 8).

Fragmentation

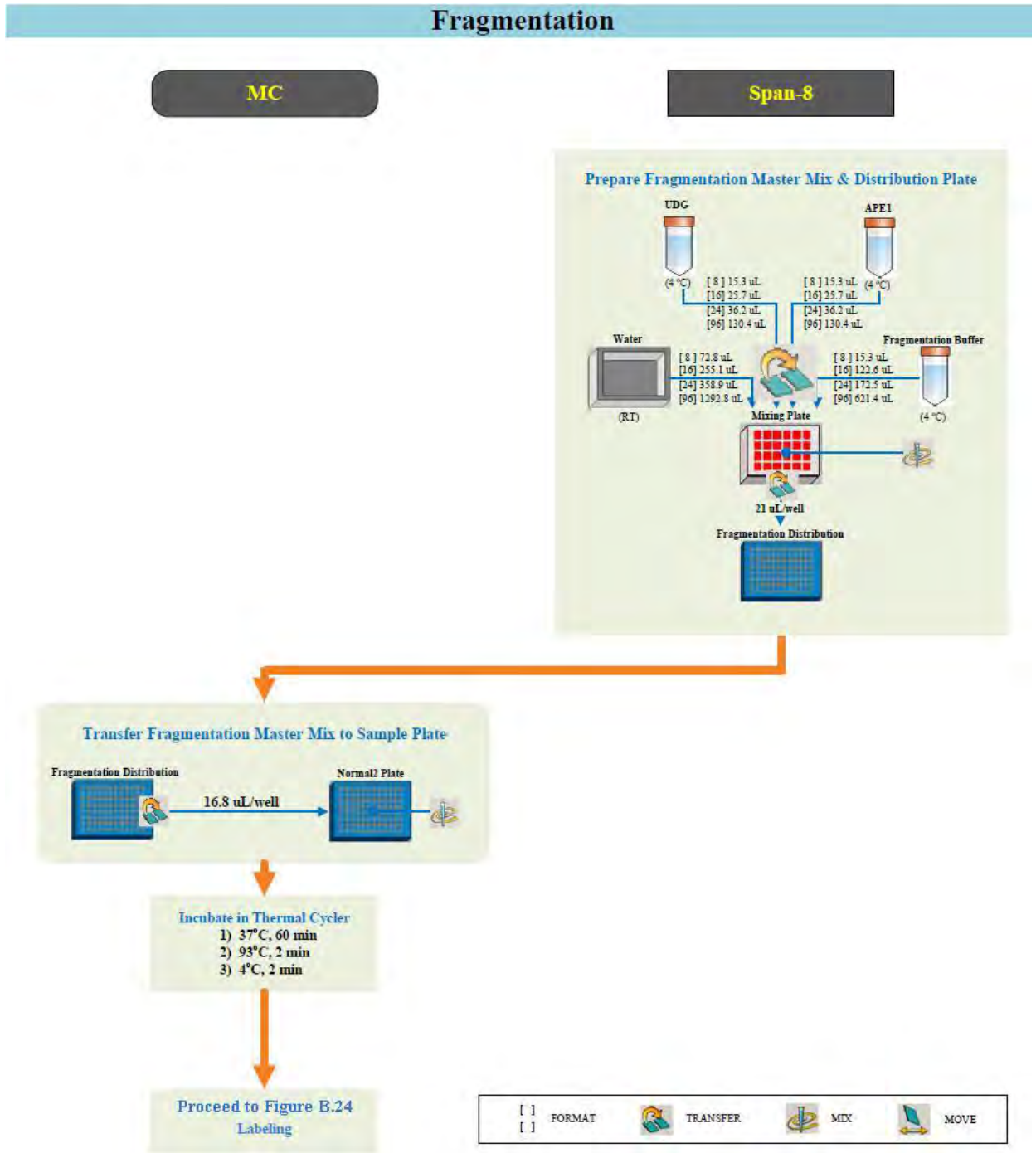


Figure 101 Fragmentation.

Labeling

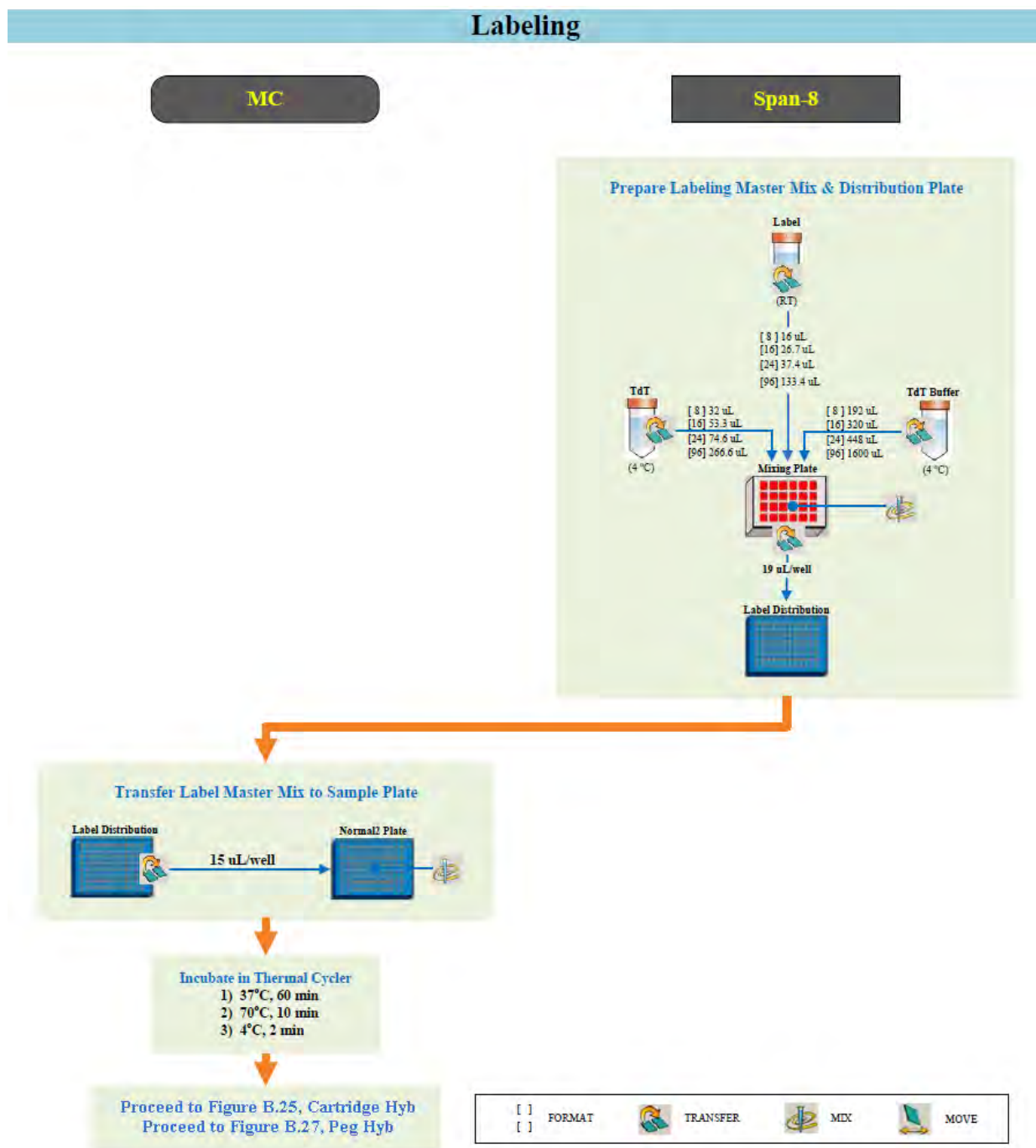


Figure 102 Labeling.

Cartridge hybridization setup

Cartridge hybridization setup (diagram 1 of 2)

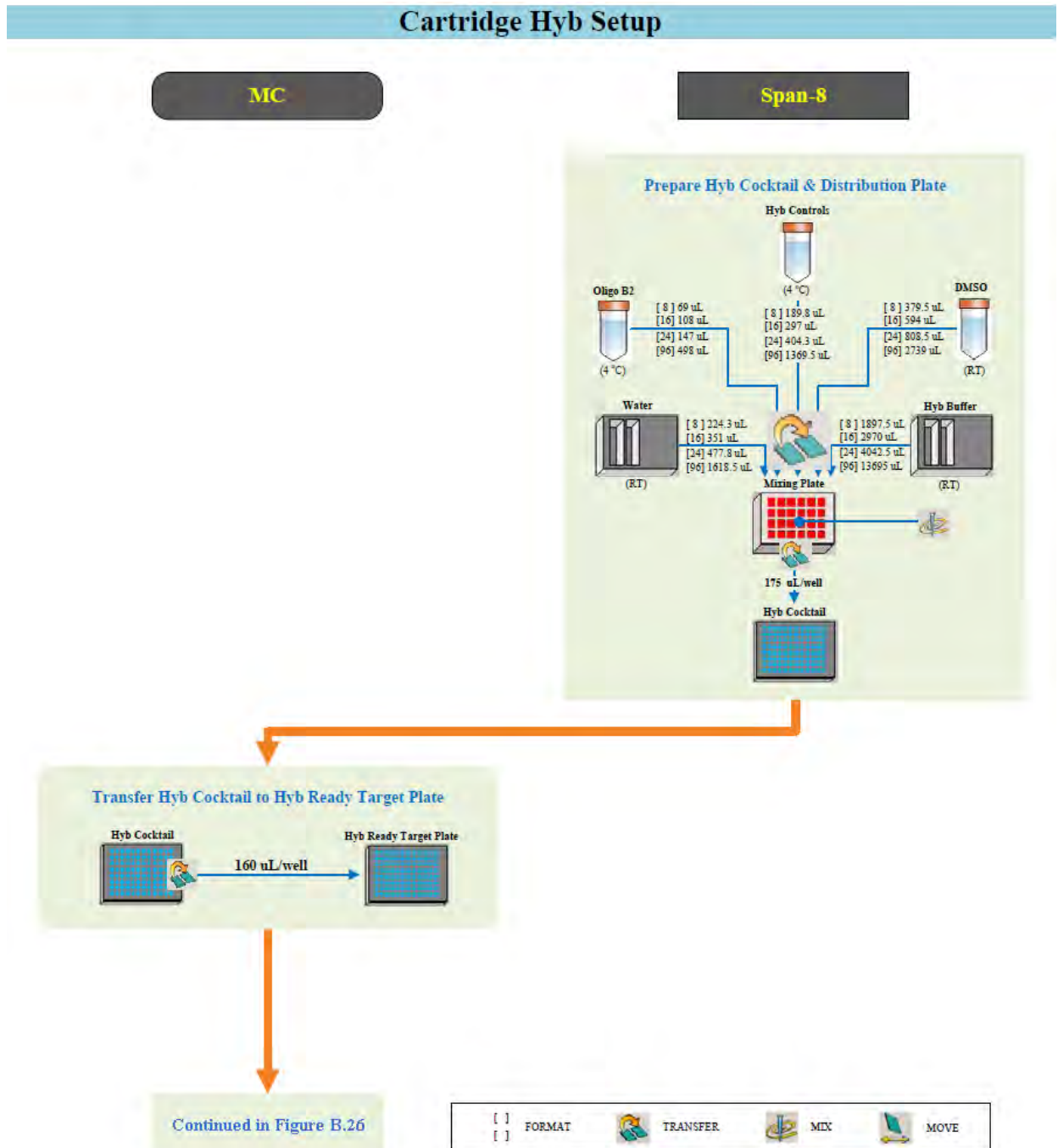


Figure 103 Cartridge hybridization setup (1 of 2).

Cartridge hybridization setup (diagram 2 of 2)

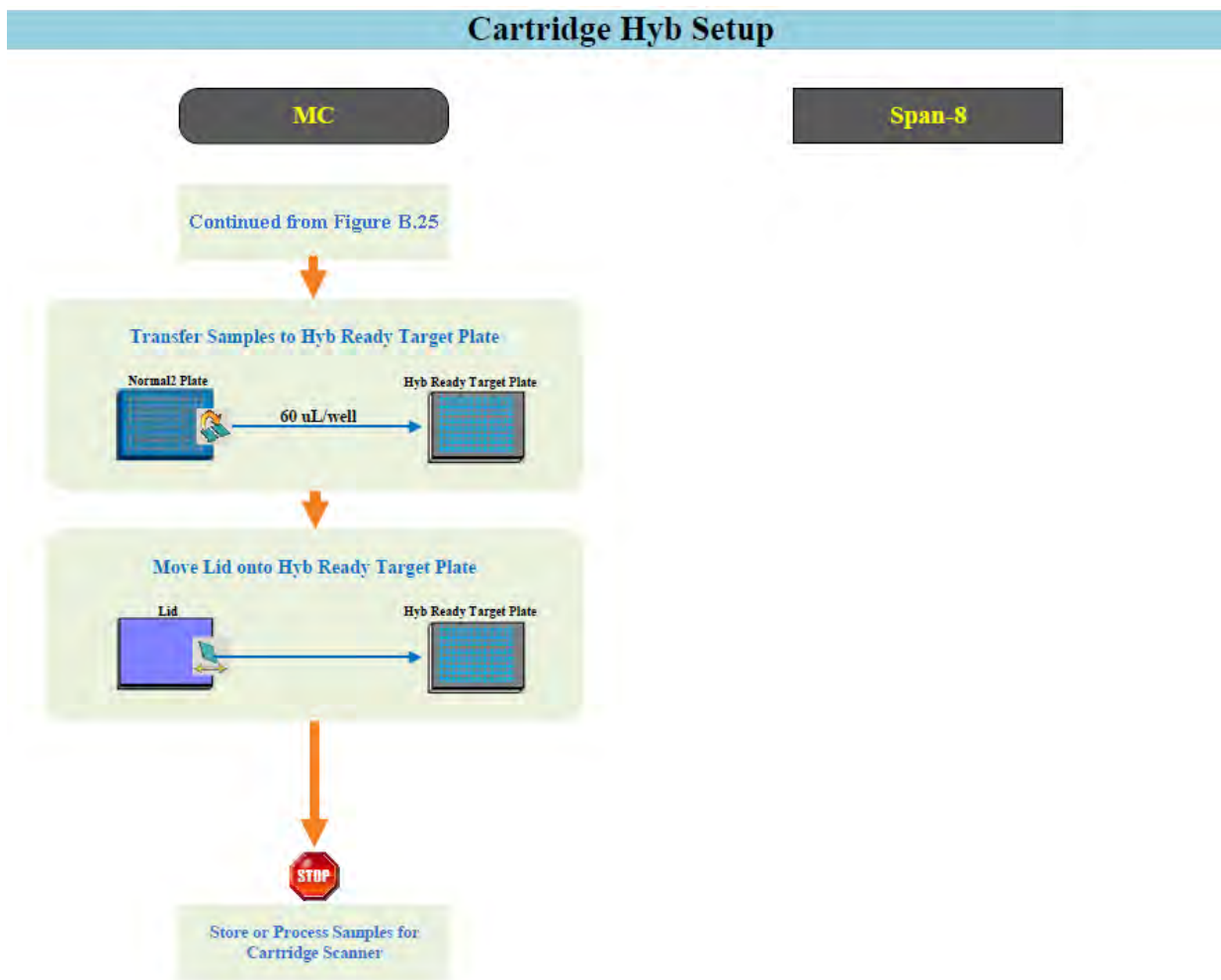


Figure 104 Cartridge hybridization setup (2 of 2).

Array plate hybridization setup

Array plate hybridization setup (diagram 1 of 2)

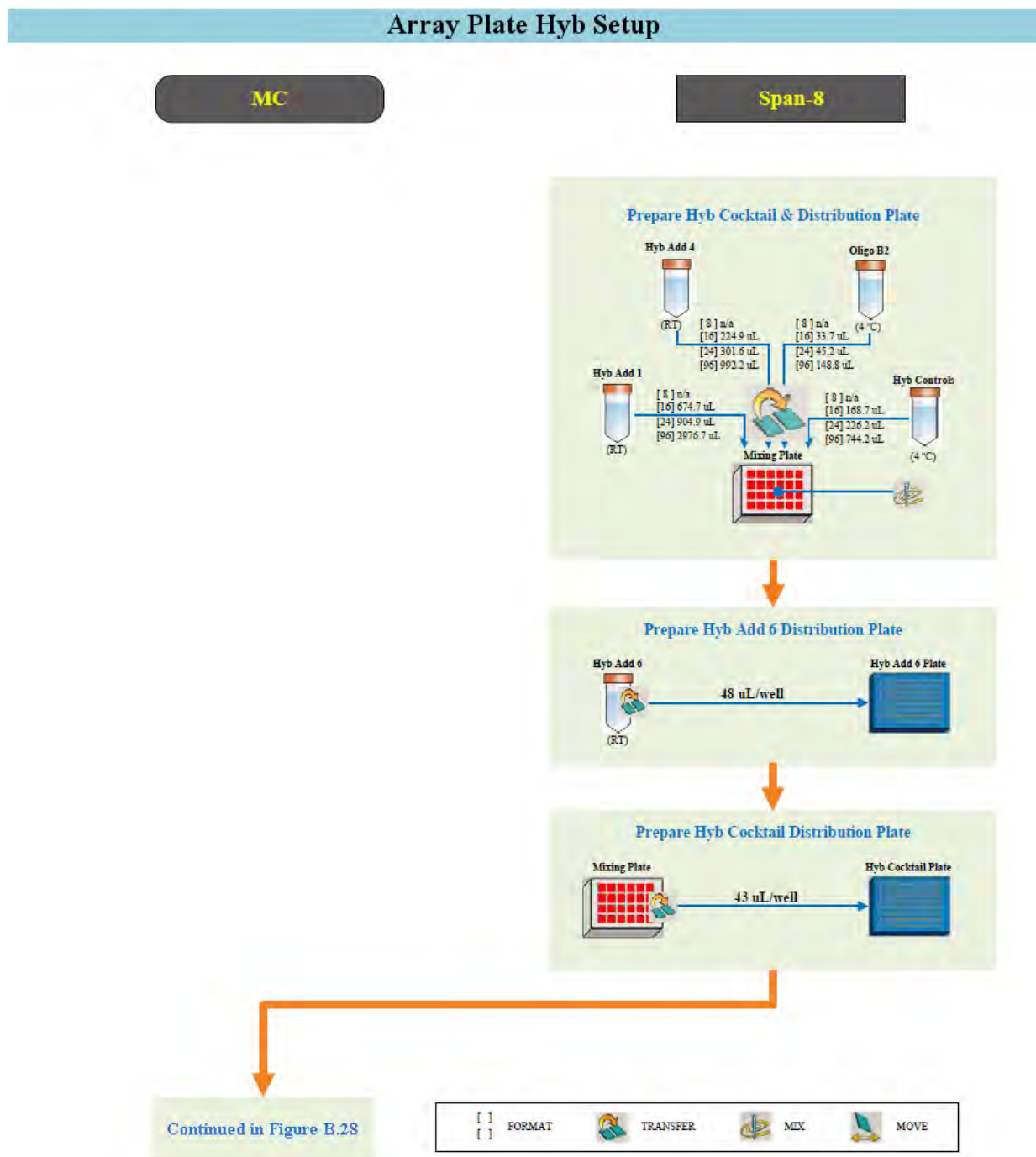


Figure 105 Array plate hybridization setup (1 of 2).

Array plate hybridization setup (diagram 2 of 2)

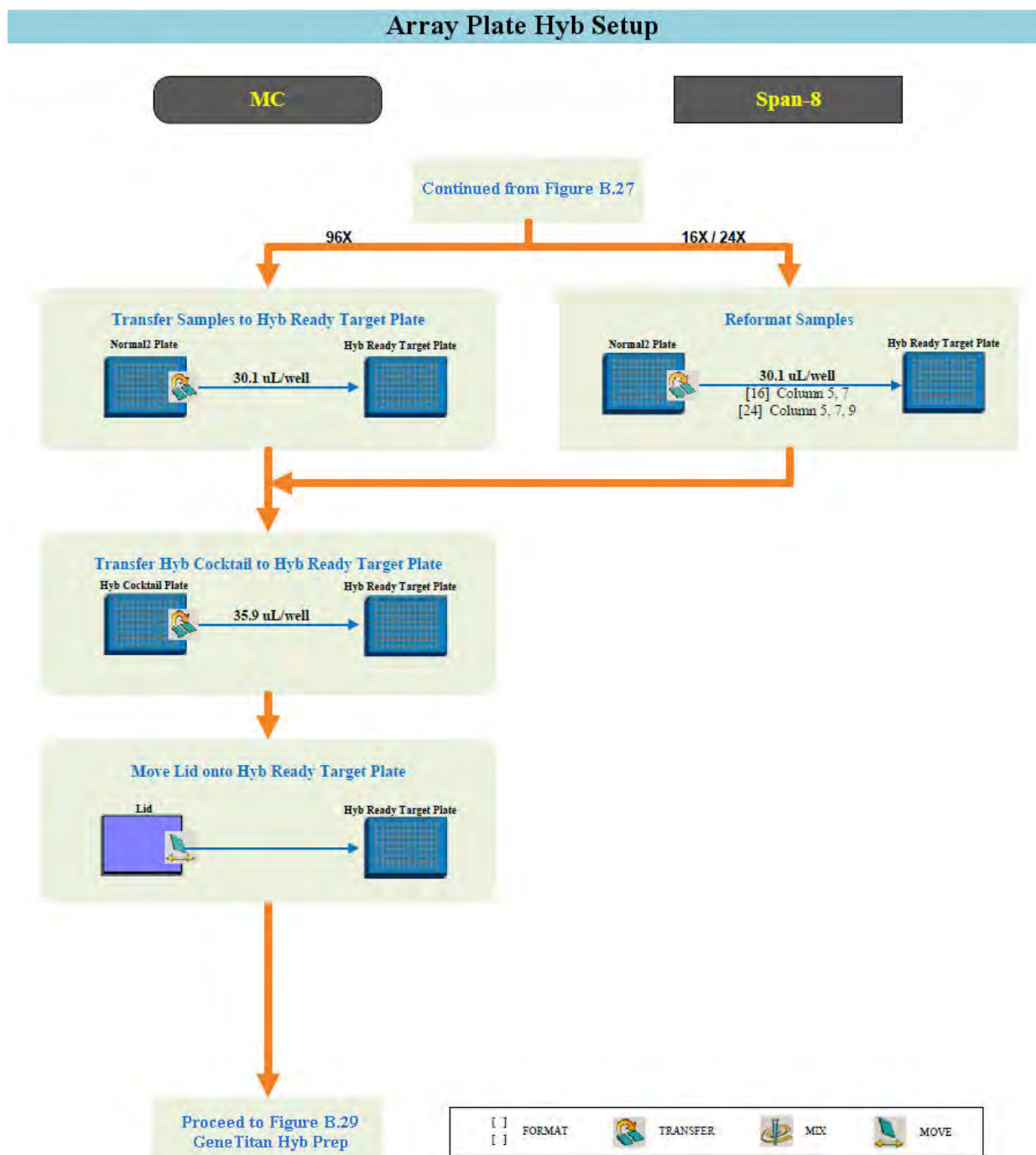


Figure 106 Array plate hybridization setup (2 of 2).

GeneTitan™ MC Instrument preparation

GeneTitan™ MC Instrument preparation (diagram 1 of 2)

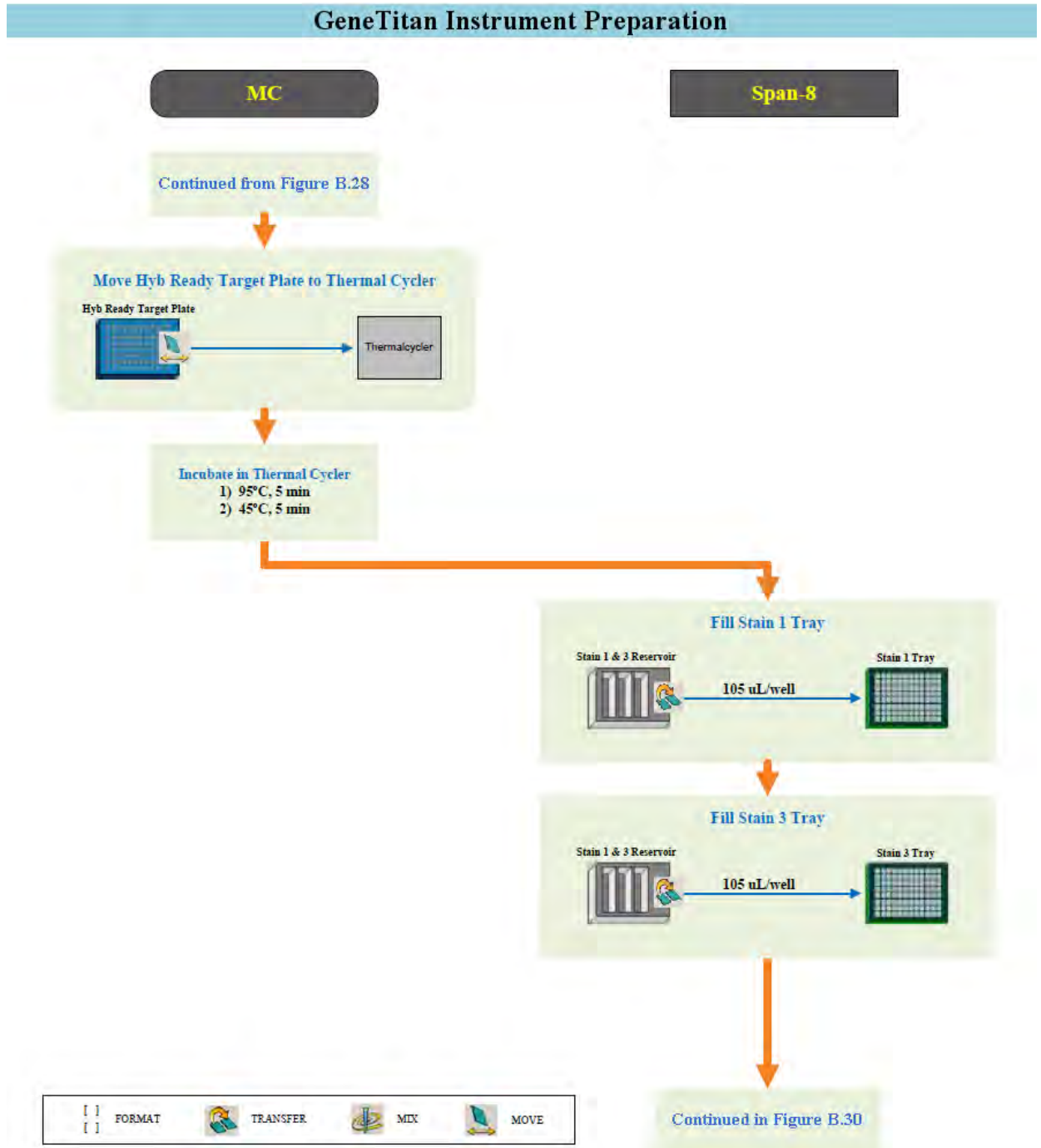


Figure 107 GeneTitan™ MC Instrument preparation (1 of 2).

GeneTitan™ MC Instrument preparation (diagram 2 of 2)

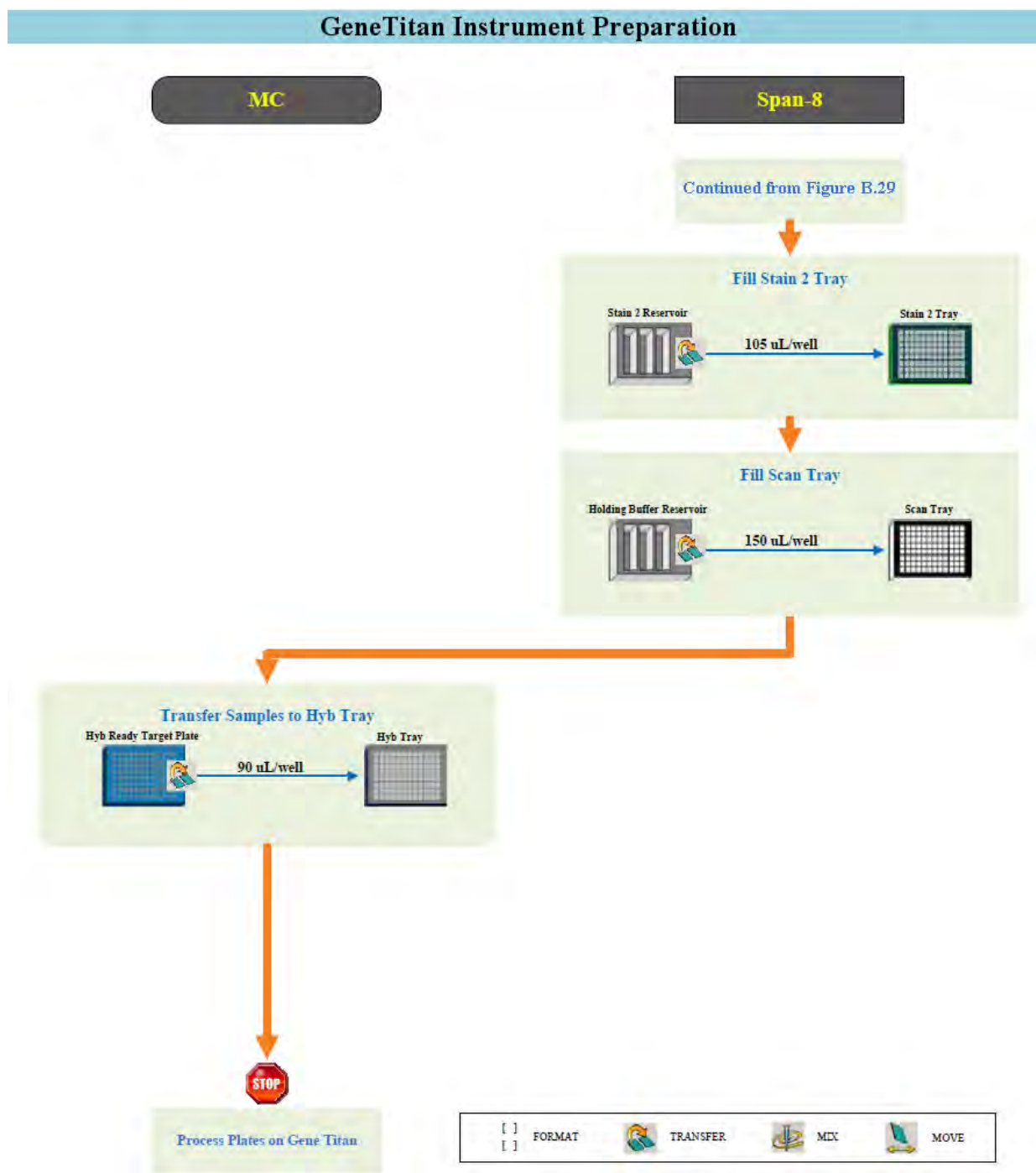


Figure 108 GeneTitan™ MC Instrument preparation (2 of 2).



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.



- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.pdf>
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
<i>GeneTitan™ Instrument User Guide</i>	08-0296
<i>GeneTitan™ Multi-Channel Instrument User Guide</i>	08-0308
<i>GeneTitan™ Instrument User Guide for Expression Array Plates</i>	702933
<i>GeneChip™ Expression Wash, Stain, and Scan for Expression Cartridge Arrays User Guide</i>	MAN0018114
<i>Biomek™ Liquid Handler User's Manual</i>	987834
<i>Biomek™ Software User's Manual</i>	987835

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 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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